10/510002

10 Rec'd PCT/PTO 30 SEP 2004

XCRF POLYNUCLEOTIDES AND POLYPEPTIDES AND USES THEREOF

FIELD OF THE INVENTION

The present invention relates to the field of metabolic research, in particular the discovery of compounds effective for reducing body mass and useful for treating metabolic-related diseases and disorders. The metabolic-related diseases or disorders envisioned to be treated by the methods of the invention include, but are not limited to, hyperlipidemia, atherosclerosis, diabetes, and hypertension.

BACKGROUND OF THE INVENTION

Obesity is a public health problem that is serious, widespread, and increasing. In the United States, 20 percent of the population is obese; in Europe, a slightly lower percentage is obese (Friedman (2000) Nature 404:632-634). Obesity is associated with increased risk of hypertension, cardiovascular disease, diabetes, and cancer as well as respiratory complications and osteoarthritis (Kopelman (2000) Nature 404:635-643). Even modest weight loss ameliorates these associated conditions.

Maintenance of weight gain or loss is associated with compensatory changes in energy expenditure that oppose the maintenance of a body weight that is different from the usual weight [Leibel et al. (1995) N Engl J Med 332:621–8]. These changes may account, in part, for the poor long-term efficacy of obesity treatments [Wadden (1993) Ann Intern Med 229:688–93]. Further, the decreased insulin sensitivity after weight gain and the beneficial effects of even modest amounts of weight reduction on carbohydrate metabolism and insulin sensitivity in some patients are well documented [Olefsky et al. (1974) J Clin Invest 53:64–76].

While still acknowledging that lifestyle factors including environment, diet, age and exercise play a role in obesity, twin studies, analyses of familial aggregation, and adoption studies all indicate that obesity is largely the result of genetic factors (Barsh et al (2000) Nature 404:644-651). In agreement with these studies, is the fact that an increasing number of metabolic-related genes are being identified. Some of the more extensively studied genes include those encoding leptin (*ob*) and its receptor (*db*), pro-opiomelanocortin (*Pomc*), melanocortin-4-receptor (*Mc4r*), agouti protein (A^y), carboxypeptidase E (*fat*), 5-hydroxytryptamine receptor 2C (*Htr2c*), nescient basic helix-loop-helix 2 (*Nhlh2*), prohormone convertase 1 (PCSK1), and tubby protein (*tubby*) (rev'd in Barsh et al (2000) Nature 404:644-651).

SUMMARY OF THE INVENTION

The instant invention is based on XCRF1, XCRF3, XCRF4 and XCRD5, which may in the following be commonly designated "XCRF" or "XCRFs". XCRFs are of human origin. XCRF full-length polypeptide is comprised of a globular C-terminal C1q homology domain preceded by a

collagen-like region, an N-terminal unique region, and a putative signal peptide. XCRF polypeptide is able to lower circulating (either blood, serum, or plasma) levels (concentration) of: (i) free fatty acids, (ii) glucose, and/or (iii) triglycerides. XCRF polypeptide is further able to (i) prevent weight gain, (ii) reduce weight, and/or (iii) maintain weight loss.

In particular, the invention relates to the use of XCRF for the preparation of a medicament for treating and/or preventing a metabolic disease, wherein XCRF is selected from

a) A polypeptide comprising SEQ ID NO: 2;
b) A polypeptide comprising amino acids 17 to 258 of SEQ ID NO: 2;
c) A polypeptide comprising SEQ ID NO: 4;
d) A polypeptide comprising amino acids 22 to 287 of SEQ ID NO: 4;
e) A polypeptide comprising SEQ ID NO: 6;
f) A polypeptide comprising amino acids 16 to 238 of SEQ ID NO: 6;
g) A polypeptide comprising SEQ ID NO: 8;
h) A polypeptide comprising amino acids 22 to 287 of SEQ ID NO: 8;
i) A fragment of any of (a) to (h) comprising the C-terminal C1q homology domain;
j) A mutein of any of (a) to (i), wherein the amino acid sequence has at least 40 % or 50 % or 60 % or 70 % or 80 % or 90 % identity to at least one of the sequences in (a) to (i);
k) A mutein of any of (a) to (i) which is encoded by a DNA sequence which hybridizes to the complement of the native DNA sequence encoding any of (a) to (i) under moderately stringent conditions or under highly stringent conditions;
l) A mutein of any of (a) to (i) wherein any changes in the amino acid sequence are conservative amino acid substitutions to the amino acid sequences in (a) to (i);
m) a salt or an isoform, fused protein, functional derivative, active fraction or circularly permutated derivative of any of (a) to (i).

In a preferred embodiment, said metabolic disease is selected from the group consisting of:

a) obesity;
b) impaired glucose tolerance;
c) insulin resistance;
d) Syndrome X;
e) atherosclerosis; and
f) Type II diabetes.

In a further preferred embodiment, the XCRF is glycosylated at one or more sites.

In an alternative embodiment, the substance is not glycosylated.

The fused protein may comprise an immunoglobulin (Ig) fusion.

The functional derivative may comprise at least one moiety attached to one or more functional groups which occur as one or more side chains on the amino acid residues.

In a preferred embodiment, the moiety is a polyethylene moiety.

In a further aspect, the invention relates to the use of a nucleic acid molecule for manufacture of a medicament for the treatment and/or prevention of a metabolic disease, wherein the nucleic acid molecule comprises a nucleic acid sequence encoding a polypeptide selected from:

a) A polypeptide comprising SEQ ID NO: 2;
b) A polypeptide comprising amino acids 17 to 258 of SEQ ID NO: 2;
c) A polypeptide comprising SEQ ID NO: 4;
d) A polypeptide comprising amino acids 22 to 287 of SEQ ID NO: 4;
e) A polypeptide comprising SEQ ID NO: 6;
f) A polypeptide comprising amino acids 16 to 238 of SEQ ID NO: 6;
g) A polypeptide comprising SEQ ID NO: 8;
h) A polypeptide comprising amino acids 22 to 287 of SEQ ID NO: 8;
i) A fragment of any of (a) to (h) comprising the C-terminal C1q homology domain;
j) A mutein of any of (a) to (i), wherein the amino acid sequence has at least 40 % or 50 % or 60 % or 70 % or 80 % or 90 % identity to at least one of the sequences in (a) to (i);
k) A mutein of any of (a) to (i) which is encoded by a DNA sequence which hybridizes to the complement of the native DNA sequence encoding any of (a) to (i) under moderately stringent conditions or under highly stringent conditions;
l) A mutein of any of (a) to (i) wherein any changes in the amino acid sequence are conservative amino acid substitutions to the amino acid sequences in (a) to (i);
m) an isoform, fused protein, active fraction or circularly permutated derivative of any of (a) to (i).

In a further aspect, the invention relates to the use of a vector comprising said nucleic acid molecule for the manufacture of a medicament for treatment and/or prevention of a metabolic disease.

The vector may preferably be an expression vector. It may further be a gene therapy vector.

Use of a vector for inducing and/or enhancing the endogenous production of a polypeptide according to any of claims 2 to 5 in a cell for the preparation of a medicament for the treatment and/or prevention of a metabolic disorder.

In yet a further aspect, the invention relates to the use of a cell comprising a vector according to the invention for the preparation of a medicament for the treatment and/or prevention of a metabolic disorder. Preferably, the metabolic diseases is selected from:

a) obesity;

b) impaired glucose tolerance;
c) insulin resistance;
d) Syndrome X;
e) atherosclerosis; and
f) Type II diabetes.

The invention includes polypeptides encoded by XCRFs, which include both the full-length polypeptide and fragments thereof, preferably said polypeptide fragments comprising all or part of the globular C-terminal C1q homology domain. The XCRF polypeptide fragments containing all or part of the globular C-terminal C1q homology domain have *in vitro* and *in vivo* biological activity as described herein, including utility for weight reduction, prevention of weight gain and control of blood glucose levels in humans and other mammals. More specifically, the biological activities of the XCRF polypeptides, including fragments, include reduction of elevated free fatty acid levels caused by administration of epinephrine, *i.v.* injection of "intralipid", or administration of a high fat test meal, as well as increased fatty acid oxidation in muscle cells, reduction in glucose levels, modulation of energy expenditure, prevention and/or reduction of insulin resistance, and weight reduction in mammals consuming a high fat/high sucrose diet.

Thus, the invention is drawn to XCRF polypeptides, polynucleotides encoding said XCRF polypeptides, vectors comprising said XCRF polynucleotides, and cells recombinant for said XCRF polynucleotides, as well as to pharmaceutical and physiologically acceptable compositions comprising said XCRF polypeptides and methods of administering said XCRF pharmaceutical and physiologically acceptable compositions in order to reduce body weight or to treat metabolic-related diseases and disorders. Assays for identifying agonists and antagonists of metabolic-related activity are also part of the invention.

Antagonists of XCRF polypeptide activity should be effective in the treatment of other metabolic-related diseases or disorders of the invention including cachexia, wasting, AIDS-related weight loss, cancer-related weight loss, anorexia, and bulimia. In preferred embodiments, said individual is a mammal, preferably a human.

In a first aspect, the invention features purified, isolated, or recombinant XCRF1 polypeptides that have lipid partitioning, lipid metabolism, and insulin-like activities. Preferred XCRF polypeptide fragments are said polypeptide fragments having activity, wherein said activity is also selected from the group consisting of lipid partitioning, lipid metabolism, and insulin-like activity. In preferred embodiments, said polypeptide fragment comprises, consists essentially of, or consists of, at least 6 consecutive amino acids and not more than 258 consecutive amino acids of SEQ ID NO: 2.

In a second aspect, the invention features purified, isolated, or recombinant XCRF3 polypeptides that have lipid partitioning, lipid metabolism, and insulin-like activities. Preferred XCRF polypeptide fragments are said polypeptide fragments having activity, wherein said activity is also selected from the group consisting of lipid partitioning, lipid metabolism, and insulin-like activity. In preferred embodiments, said polypeptide fragment comprises, consists essentially of, or consists of, at least 6 consecutive amino acids and not more than 287 consecutive amino acids of SEQ ID NO: 4.

In a third aspect, the invention features purified, isolated, or recombinant XCRF4 polypeptides that have lipid partitioning, lipid metabolism, and insulin-like activities. Preferred XCRF4 polypeptide fragments are said polypeptide fragments having activity, wherein said activity is also selected from the group consisting of lipid partitioning, lipid metabolism, and insulin-like activity. In preferred embodiments, said polypeptide fragment comprises, consists essentially of, or consists of, at least 6 consecutive amino acids and not more than 238 consecutive amino acids of SEQ ID NO: 6.

In a fourth aspect, the invention features purified, isolated, or recombinant XCRF5 polypeptides that have lipid partitioning, lipid metabolism, and insulin-like activities. Preferred XCRF5 polypeptide fragments are said polypeptide fragments having activity, wherein said activity is also selected from the group consisting of lipid partitioning, lipid metabolism, and insulin-like activity. In preferred embodiments, said polypeptide fragment comprises, consists essentially of, or consists of, at least 6 consecutive amino acids and not more than 287 consecutive amino acids of SEQ ID NO: 8.

In other preferred embodiments, XCRF1 polypeptide fragments having activity are selected from amino acids 17-258, 18-258, 19-258, 20-258, 21-258, 22-258, 23-258, 24-258, 25-258, 26-258, 27-258, 28-258, 29-258, 30-258, 31-258, 32-258, 33-258, 34-258, 35-258, 36-258, 37-258, 38-258, 39-258, 40-258, 41-258, 42-258, 43-258, 44-258, 45-258, 46-258, 47-258, 48-258, 49-258, 50-258, 51-258, 52-258, 53-258, 54-258, 55-258, 56-258, 57-258, 58-258, 59-258, 60-258, 61-258, 62-258, 63-258, 64-258, 65-258, 66-258, 67-258, 68-258, 69-258, 70-258, 71-258, 72-258, 73-258, 74-258, 75-258, 76-258, 77-258, 78-258, 79-258, 80-258, 81-258, 82-258, 83-258, 84-258, 85-258, 86-258, 87-258, 88-258, 89-258, 90-258, 91-258, 92-258, 93-258, 94-258, 95-258, 96-258, 97-258, 98-258, 99-258, 100-258, 101-258, 102-258, 103-258, 104-258, 105-258, 106-258, 107-258, 108-258, 109-258, 110-258, 111-258, 112-258, 113-258, 114-258, 115-258, 116-258, 117-258, 118-258, 119-258, 120-258, 121-258, 122-258, 123-258, 124-258, 125-258, 126-258, 127-258, 128-258, 129-258 or 130-258 of SEQ ID NO: 2, where it is understood that amino acid 17 is taken to represent the N-terminal amino acid of mature XCRF polypeptide absent the signal peptide.

In more preferred embodiments, XCRF1 polypeptide fragments having activity are selected from amino acids 17-258, 30-258, 39-258, 46-258, 72-258, 75-258, 78-258, 99-258, 100-258, 105-258, 110-258, 115-258, 118-258, 119-258, 120-258, 121-258, 122-258, 123-258, 124-258, 125-258, 126-258,

127-258, 128-258, 129-258 or 130-258 of SEQ ID NO: 2, where it is understood that amino acid 17 is taken to represent the N-terminal amino acid of mature XCRF1 polypeptide absent the signal peptide.

In yet more preferred embodiments, XCRF1 polypeptide fragments having activity are selected from amino acids 17-258, 99-258, 100-258, 105-258, 110-258, 115-258, 118-258, 119-258, 120-258, 121-258, 122-258, 123-258, 124-258, 125-258, 126-258, 127-258, 128-258, 129-258 or 130-258 of SEQ ID NO: 2, where it is understood that amino acid 17 is taken to represent the N-terminal amino acid of mature XCRF1 polypeptide absent the signal peptide.

In further preferred embodiments, said polypeptide fragment comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the corresponding consecutive amino acids of the polypeptide sequences identified in SEQ ID NO: 2.

The invention further provides purified, isolated, or recombinant XCRF1 polypeptides that have weight reduction activities. Preferred XCRF1 polypeptide fragments are said polypeptide fragments having activity, wherein said activity is also selected from the group consisting of prevention of weight gain, weight reduction, and maintenance of weight loss. In preferred embodiments, said polypeptide fragment comprises, consists essentially of, or consists of, at least 6 consecutive amino acids and not more than 258 consecutive amino acids of SEQ ID NO: 2.

In other preferred embodiments, XCRF1 polypeptide fragments having activity are selected from amino acids 17-258, 18-258, 19-258, 20-258, 21-258, 22-258, 23-258, 24-258, 25-258, 26-258, 27-258, 28-258, 29-258, 30-258, 31-258, 32-258, 33-258, 34-258, 35-258, 36-258, 37-258, 38-258, 39-258, 40-258, 41-258, 42-258, 43-258, 44-258, 45-258, 46-258, 47-258, 48-258, 49-258, 50-258, 51-258, 52-258, 53-258, 54-258, 55-258, 56-258, 57-258, 58-258, 59-258, 60-258, 61-258, 62-258, 63-258, 64-258, 65-258, 66-258, 67-258, 68-258, 69-258, 70-258, 71-258, 72-258, 73-258, 74-258, 75-258, 76-258, 77-258, 78-258, 79-258, 80-258, 81-258, 82-258, 83-258, 84-258, 85-258, 86-258, 87-258, 88-258, 89-258, 90-258, 91-258, 92-258, 93-258, 94-258, 95-258, 96-258, 97-258, 98-258, 99-258, 100-258, 101-258, 102-258, 103-258, 104-258, 105-258, 106-258, 107-258, 108-258, 109-258, 110-258, 111-258, 112-258, 113-258, 114-258, 115-258, 116-258, 117-258, 118-258, 119-258, 120-258, 121-258, 122-258, 123-258, 124-258, 125-258, 126-258, 127-258, 128-258, 129-258 or 130-258 of SEQ ID NO: 2, where it is understood that amino acid 17 is taken to represent the N-terminal amino acid of mature XCRF polypeptide absent the signal peptide.

In more preferred embodiments, XCRF1 polypeptide fragments having activity are selected from amino acids 17-258, 30-258, 39-258, 46-258, 72-258, 75-258, 78-258, 99-258, 100-258, 105-258, 110-258, 115-258, 118-258, 119-258, 120-258, 121-258, 122-258, 123-258, 124-258, 125-258, 126-258, 127-258, 128-258, 129-258 or 130-258 of SEQ ID NO: 2, where it is understood that amino acid 17 is taken to represent the N-terminal amino acid of mature XCRF1 polypeptide absent the signal peptide.

In yet more preferred embodiments, XCRF1 polypeptide fragments having activity are selected from amino acids 17-258, 99-258, 100-258, 105-258, 110-258, 115-258, 118-258, 119-258, 120-258, 121-258, 122-258, 123-258, 124-258, 125-258, 126-258, 127-258, 128-258, 129-258 or 130-258 of SEQ ID NO: 2, where it is understood that amino acid 17 is taken to represent the N-terminal amino acid of mature XCRF1 polypeptide absent the signal peptide.

In further preferred embodiments, said polypeptide fragment comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the corresponding consecutive amino acids of the polypeptide sequences identified in SEQ ID NO: 2.

The invention yet further provides a purified or isolated polypeptide comprising, consisting of, or consisting essentially of an amino acid sequence selected from the group consisting of: (a) a full-length XCRF polypeptide at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the corresponding polypeptide of SEQ ID NO: 2; (b) a full-length XCRF1 polypeptide of SEQ ID NO: 2 absent the N-terminal Met; (c) a mature XCRF1 polypeptide of SEQ ID NO: 2 lacking signal peptide; (d) an XCRF1 polypeptide of SEQ ID NO: 2 wherein said XCRF1 polypeptide is of any one integer in length between 6 amino acids and 258 amino acids (full-length) inclusive of SEQ ID NO: 2; (e) the epitope-bearing fragments of an XCRF1 polypeptide of SEQ ID NO: 2; (f) the allelic variant polypeptides of any of the polypeptides of (a)-(e). The invention further provides for fragments of the polypeptides of (a)-(f) above, such as those having biological activity or comprising biologically functional domain(s).

In other highly preferred embodiments, XCRF1 polypeptides comprise, consist essentially of, or consist of, a purified, isolated, or a recombinant XCRF1 polypeptide fragment comprised of all or part of the globular C-terminal C1q homology domain. Preferably, said XCRF1 polypeptide fragment comprises, consists essentially of, or consists of, at least 6 consecutive amino acids of amino acids 17-258 of SEQ ID NO: 2, where it is understood that amino acid 17 is taken to represent the N-terminal amino acid of mature XCRF1 polypeptide absent the signal peptide. In other preferred embodiments, said XCRF1 polypeptide fragments comprising all or part of the globular C-terminal C1q homology domain are selected from amino acids 17-258, 18-258, 19-258, 20-258, 21-258, 22-258, 23-258, 24-258, 25-258, 26-258, 27-258, 28-258, 29-258, 30-258, 31-258, 32-258, 33-258, 34-258, 35-258, 36-258, 37-258, 38-258, 39-258, 40-258, 41-258, 42-258, 43-258, 44-258, 45-258, 46-258, 47-258, 48-258, 49-258, 50-258, 51-258, 52-258, 53-258, 54-258, 55-258, 56-258, 57-258, 58-258, 59-258, 60-258, 61-258, 62-258, 63-258, 64-258, 65-258, 66-258, 67-258, 68-258, 69-258, 70-258, 71-258, 72-258, 73-258, 74-258, 75-258, 76-258, 77-258, 78-258, 79-258, 80-258, 81-258, 82-258, 83-258, 84-258, 85-258, 86-258, 87-258, 88-258, 89-258, 90-258, 91-258, 92-258, 93-258, 94-258, 95-258, 96-258, 97-258, 98-258, 99-258, 100-258, 101-258, 102-258, 103-258, 104-258, 105-258, 106-258, 107-258, 108-258, 109-258, 110-258,

111-258, 112-258, 113-258, 114-258, 115-258, 116-258, 117-258, 118-258, 119-258, 120-258, 121-258, 122-258, 123-258, 124-258, 125-258, 126-258, 127-258, 128-258, 129-258 or 130-258 of SEQ ID NO: 2, where it is understood that amino acid 17 is taken to represent the N-terminal amino acid of mature XCRF1 polypeptide absent the signal peptide.

In more preferred embodiments, XCRF1 polypeptide fragments comprising all or part of the globular C-terminal C1q homology domain are selected from amino acids 17-258, 30-258, 39-258, 46-258, 72-258, 75-258, 78-258, 99-258, 100-258, 105-258, 110-258, 115-258, 118-258, 119-258, 120-258, 121-258, 122-258, 123-258, 124-258, 125-258, 126-258, 127-258, 128-258, 129-258 or 130-258 of SEQ ID NO: 2, where it is understood that amino acid 17 is taken to represent the N-terminal amino acid of mature XCRF1 polypeptide absent the signal peptide.

In yet more preferred embodiments, XCRF1 polypeptide fragments comprising all or part of the globular C-terminal C1q homology domain are selected from amino acids 17-258, 99-258, 100-258, 105-258, 110-258, 115-258, 118-258, 119-258, 120-258, 121-258, 122-258, 123-258, 124-258, 125-258, 126-258, 127-258, 128-258, 129-258 or 130-258 of SEQ ID NO: 2, where it is understood that amino acid 17 is taken to represent the N-terminal amino acid of mature XCRF1 polypeptide absent the signal peptide.

Alternatively, said XCRF1 polypeptide fragment comprises, consists essentially of, or consists of, an amino acid sequence at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the corresponding amino acids 118-258 of SEQ ID NO: 2.

In a further aspect, the invention features purified, isolated, or recombinant XCRF3 polypeptides that have lipid partitioning, lipid metabolism, and insulin-like activities. Preferred XCRF3 polypeptide fragments are said polypeptide fragments having activity, wherein said activity is also selected from the group consisting of lipid partitioning, lipid metabolism, and insulin-like activity. In preferred embodiments, said polypeptide fragment comprises, consists essentially of, or consists of, at least 6 consecutive amino acids and not more than 287 consecutive amino acids of SEQ ID NO: 4.

In other preferred embodiments, XCRF3 polypeptide fragments having activity are selected from amino acids 22-287, 23-287, 24-287, 25-287, 26-287, 27-287, 28-287, 29-287, 30-287, 31-287, 32-287, 33-287, 34-287, 35-287, 36-287, 37-287, 38-287, 39-287, 40-287, 41-287, 42-287, 43-287, 44-287, 45-287, 46-287, 47-287, 48-287, 49-287, 50-287, 51-287, 52-287, 53-287, 54-287, 55-287, 56-287, 57-287, 58-287, 59-287, 60-287, 61-287, 62-287, 63-287, 64-287, 65-287, 66-287, 67-287, 68-287, 69-287, 70-287, 71-287, 72-287, 73-287, 74-287, 75-287, 76-287, 77-287, 78-287, 79-287, 80-287, 81-287, 82-287, 83-287, 84-287, 85-287, 86-287, 87-287, 88-287, 89-287, 90-287, 91-287, 92-287, 93-287, 94-287, 95-287, 96-287, 97-287, 98-287, 99-287, 100-287, 101-287, 102-287, 103-287, 104-287, 105-287, 106-287, 107-287, 108-287, 109-287, 110-287, 111-287, 112-287, 113-287, 114-287, 115-287, 116-287,

117-287, 118-287, 119-287, 120-287, 121-287, 122-287, 123-287, 124-287, 125-287, 126-287, 127-287, 128-287, 129-287, 130-287, 131-287, 132-287, 133-287, 134-287, 135-287, 136-287, 137-287, 138-287, 139-287, 140-287, 141-287, 142-287, 143-287, 144-287, 145-287, 146-287, 147-287, 148-287, 149-287, 150-287, 151-287, 152-287, 153-287, 154-287, 155-287, 156-287, 157-287, 158-287 or 159-287 of SEQ ID NO: 4, where it is understood that amino acid 22 is taken to represent the N-terminal amino acid of mature XCRF3 polypeptide absent the signal peptide.

In further preferred embodiments, said polypeptide fragment comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the corresponding consecutive amino acids of the polypeptide sequences identified in SEQ ID NO: 4. In yet further preferred embodiments, said polypeptide fragment comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the corresponding consecutive amino acids of the polypeptide sequences identified in SEQ ID NO: 4.

The invention further provides purified, isolated, or recombinant XCRF3 polypeptides that have weight reduction activities. Preferred XCRF3 polypeptide fragments are said polypeptide fragments having activity, wherein said activity is also selected from the group consisting of prevention of weight gain, weight reduction, and maintenance of weight loss. In preferred embodiments, said polypeptide fragment comprises, consists essentially of, or consists of, at least 6 consecutive amino acids and not more than 287 consecutive amino acids of SEQ ID NO: 4.

The invention yet further provides a purified or isolated polypeptide comprising, consisting of, or consisting essentially of an amino acid sequence selected from the group consisting of: (a) a full-length XCRF3 polypeptide at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the corresponding polypeptide of SEQ ID NO: 4; (b) a full-length XCRF3 polypeptide at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the corresponding polypeptide of SEQ ID NO: 4; (c) a full-length XCRF3 polypeptide of SEQ ID NO: 4 absent the N-terminal Met; (d) a mature XCRF3 polypeptide of SEQ ID NO: 4 lacking signal peptide; (e) a XCRF3 polypeptide of SEQ ID NO: 2 wherein said XCRF3 polypeptide is of any one integer in length between 6 amino acids and 287 amino acids (full-length) inclusive of SEQ ID NO: 4; (f) the epitope-bearing fragments of a XCRF3 polypeptide of SEQ ID NO: 4; (g) the allelic variant polypeptides of any of the polypeptides of (a)-(f). The invention further provides for fragments of the polypeptides of (a)-(g) above, such as those having biological activity or comprising biologically functional domain(s).

In other highly preferred embodiments, XCRF3 polypeptides comprise, consist essentially of, or consist of, a purified, isolated, or a recombinant XCRF3 polypeptide fragment comprised of all or part of the globular C-terminal C1q homology domain. Preferably, said XCRF3 polypeptide fragment comprises, consists essentially of, or consists of, at least 6 consecutive amino acids of amino acids 22-

287 of SEQ ID NO: 4, where it is understood that amino acid 22 is taken to represent the N-terminal amino acid of mature XCRF3 polypeptide absent the signal peptide. In other preferred embodiments, said XCRF3 polypeptide fragments comprising all or part of the globular C-terminal C1q homology domain are selected from amino acids 22-287, 23-287, 24-287, 25-287, 26-287, 27-287, 28-287, 29-287, 30-287, 31-287, 32-287, 33-287, 34-287, 35-287, 36-287, 37-287, 38-287, 39-287, 40-287, 41-287, 42-287, 43-287, 44-287, 45-287, 46-287, 47-287, 48-287, 49-287, 50-287, 51-287, 52-287, 53-287, 54-287, 55-287, 56-287, 57-287, 58-287, 59-287, 60-287, 61-287, 62-287, 63-287, 64-287, 65-287, 66-287, 67-287, 68-287, 69-287, 70-287, 71-287, 72-287, 73-287, 74-287, 75-287, 76-287, 77-287, 78-287, 79-287, 80-287, 81-287, 82-287, 83-287, 84-287, 85-287, 86-287, 87-287, 88-287, 89-287, 90-287, 91-287, 92-287, 93-287, 94-287, 95-287, 96-287, 97-287, 98-287, 99-287, 100-287, 101-287, 102-287, 103-287, 104-287, 105-287, 106-287, 107-287, 108-287, 109-287, 110-287, 111-287, 112-287, 113-287, 114-287, 115-287, 116-287, 117-287, 118-287, 119-287, 120-287, 121-287, 122-287, 123-287, 124-287, 125-287, 126-287, 127-287, 128-287, 129-287, 130-287, 131-287, 132-287, 133-287, 134-287, 135-287, 136-287, 137-287, 138-287, 139-287, 140-287, 141-287, 142-287, 143-287, 144-287, 145-287, 146-287, 147-287, 148-287, 149-287, 150-287, 151-287, 152-287, 153-287, 154-287, 155-287, 156-287, 157-287, 158-287 or 159-287 of SEQ ID NO: 4, where it is understood that amino acid 22 is taken to represent the N-terminal amino acid of mature XCRF3 polypeptide absent the signal peptide.

In other highly preferred embodiments, XCRF3 polypeptides comprise, consist essentially of, or consist of, a purified, isolated, or a recombinant XCRF3 polypeptide fragment comprised of all or part of the globular C-terminal C1q homology domain and having ability to bind to cells and thereby lead to dephosphorylation of protein kinase C alpha within said cells. Preferably, said XCRF3 polypeptide fragment comprises, consists essentially of, or consists of, at least 6 consecutive amino acids of amino acids 22-287 of SEQ ID NO: 4, where it is understood that amino acid 22 is taken to represent the N-terminal amino acid of mature XCRF3 polypeptide absent the signal peptide. In other preferred embodiments, said XCRF3 polypeptide fragments comprising all or part of the globular C-terminal C1q homology domain and having ability to bind to cells and thereby lead to dephosphorylation of protein kinase C alpha within said cells are selected from amino acids 22-287, 23-287, 24-287, 25-287, 26-287, 27-287, 28-287, 29-287, 30-287, 31-287, 32-287, 33-287, 34-287, 35-287, 36-287, 37-287, 38-287, 39-287, 40-287, 41-287, 42-287, 43-287, 44-287, 45-287, 46-287, 47-287, 48-287, 49-287, 50-287, 51-287, 52-287, 53-287, 54-287, 55-287, 56-287, 57-287, 58-287, 59-287, 60-287, 61-287, 62-287, 63-287, 64-287, 65-287, 66-287, 67-287, 68-287, 69-287, 70-287, 71-287, 72-287, 73-287, 74-287, 75-287, 76-287, 77-287, 78-287, 79-287, 80-287, 81-287, 82-287, 83-287, 84-287, 85-287, 86-287, 87-287, 88-287, 89-287, 90-287, 91-287, 92-287, 93-287, 94-287, 95-287, 96-287, 97-287, 98-287, 99-287, 100-287, 101-287, 102-287, 103-287, 104-287, 105-287, 106-287, 107-287, 108-287, 109-287, 110-287, 111-287,

112-287, 113-287, 114-287, 115-287, 116-287, 117-287, 118-287, 119-287, 120-287, 121-287, 122-287, 123-287, 124-287, 125-287, 126-287, 127-287, 128-287, 129-287, 130-287, 131-287, 132-287, 133-287, 134-287, 135-287, 136-287, 137-287, 138-287, 139-287, 140-287, 141-287, 142-287, 143-287, 144-287, 145-287, 146-287, 147-287, 148-287, 149-287, 150-287, 151-287, 152-287, 153-287, 154-287, 155-287, 156-287, 157-287, 158-287 or 159-287 of SEQ ID NO: 4, where it is understood that amino acid 22 is taken to represent the N-terminal amino acid of mature XCRF3 polypeptide absent the signal peptide.

In other highly preferred embodiments, XCRF3 polypeptides comprise, consist essentially of, or consist of, a purified, isolated, or a recombinant XCRF3 polypeptide fragment comprised of all or part of the globular C-terminal C1q homology domain and having ability to bind to cells and thereby lead to activation of NF-kB within said cells. Preferably, said XCRF3 polypeptide fragment comprises, consists essentially of, or consists of, at least 6 consecutive amino acids of amino acids 22-287 of SEQ ID NO: 4, where it is understood that amino acid 22 is taken to represent the N-terminal amino acid of mature XCRF3 polypeptide absent the signal peptide. In other preferred embodiments, said XCRF3 polypeptide fragments comprising all or part of the globular C-terminal C1q homology domain and having ability to bind to cells and thereby lead to activation of NF-kB within said cells are selected from amino acids 22-287, 23-287, 24-287, 25-287, 26-287, 27-287, 28-287, 29-287, 30-287, 31-287, 32-287, 33-287, 34-287, 35-287, 36-287, 37-287, 38-287, 39-287, 40-287, 41-287, 42-287, 43-287, 44-287, 45-287, 46-287, 47-287, 48-287, 49-287, 50-287, 51-287, 52-287, 53-287, 54-287, 55-287, 56-287, 57-287, 58-287, 59-287, 60-287, 61-287, 62-287, 63-287, 64-287, 65-287, 66-287, 67-287, 68-287, 69-287, 70-287, 71-287, 72-287, 73-287, 74-287, 75-287, 76-287, 77-287, 78-287, 79-287, 80-287, 81-287, 82-287, 83-287, 84-287, 85-287, 86-287, 87-287, 88-287, 89-287, 90-287, 91-287, 92-287, 93-287, 94-287, 95-287, 96-287, 97-287, 98-287, 99-287, 100-287, 101-287, 102-287, 103-287, 104-287, 105-287, 106-287, 107-287, 108-287, 109-287, 110-287, 111-287, 112-287, 113-287, 114-287, 115-287, 116-287, 117-287, 118-287, 119-287, 120-287, 121-287, 122-287, 123-287, 124-287, 125-287, 126-287, 127-287, 128-287, 129-287, 130-287, 131-287, 132-287, 133-287, 134-287, 135-287, 136-287, 137-287, 138-287, 139-287, 140-287, 141-287, 142-287, 143-287, 144-287, 145-287, 146-287, 147-287, 148-287, 149-287, 150-287, 151-287, 152-287, 153-287, 154-287, 155-287, 156-287, 157-287, 158-287 or 159-287 of SEQ ID NO: 4, where it is understood that amino acid 22 is taken to represent the N-terminal amino acid of mature XCRF3 polypeptide absent the signal peptide.

In more preferred embodiments, XCRF3 polypeptide fragments comprising all or part of the globular C-terminal C1q homology domain and having ability to bind to cells and thereby lead to activation of NF-kB within said cells are selected from amino acids 22-287, 44-287, 121-287, 122-287, 123-287, 124-287, 125-287, 126-287, 127-287, 128-287, 129-287, 130-287, 131-287, 132-287, 133-287, 134-287, 135-287, 136-287, 137-287, 138-287, 139-287, 140-287, 141-287, 142-287, 143-287, 144-287,

145-287, 146-287, 147-287, 148-287, 149-287, 150-287, 151-287, 152-287, 153-287, 154-287, 155-287, 156-287, 157-287, 158-287 or 159-287 of SEQ ID NO: 4, where it is understood that amino acid 22 is taken to represent the N-terminal amino acid of mature XCRF3 polypeptide absent the signal peptide.

In yet more preferred embodiments, XCRF3 polypeptide fragments comprising all or part of the globular C-terminal C1q homology domain and having ability to bind to cells and thereby lead to activation of NF-kB within said cells are selected from amino acids 22-287, 123-287, 124-287, 125-287, 126-287, 127-287, 128-287, 129-287, 130-287, 131-287, 132-287, 133-287, 134-287, 135-287, 136-287, 137-287, 138-287, 139-287, 140-287, 141-287, 142-287, 143-287, 144-287, 145-287, 146-287, 147-287, 148-287, 149-287, 150-287, 151-287, 152-287 or 153-287 of SEQ ID NO: 4, where it is understood that amino acid 22 is taken to represent the N-terminal amino acid of mature XCRF3 polypeptide absent the signal peptide.

Alternatively, said XCRF3 polypeptide fragment comprises, consists essentially of, or consists of, an amino acid sequence at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the corresponding amino acids 121-287 of SEQ ID NO: 4.

In a further aspect, the invention features purified, isolated, or recombinant XCRF4 polypeptides that have lipid partitioning, lipid metabolism, and insulin-like activities. Preferred XCRF4 polypeptide fragments are said polypeptide fragments having activity, wherein said activity is also selected from the group consisting of lipid partitioning, lipid metabolism, and insulin-like activity. In preferred embodiments, said polypeptide fragment comprises, consists essentially of, or consists of, at least 6 consecutive amino acids and not more than 238 consecutive amino acids of SEQ ID NO: 6.

In other preferred embodiments, XCRF4 polypeptide fragments having activity are selected from amino acids 16-238, 17-238, 18-238, 19-238, 20-238, 21-238, 22-238, 23-238, 24-238, 25-238, 26-238, 27-238, 28-238, 29-238, 30-238, 31-238, 32-238, 33-238, 34-238, 35-238, 36-238, 37-238, 38-238, 39-238, 40-238, 41-238, 42-238, 43-238, 44-238, 45-238, 46-238, 47-238, 48-238, 49-238, 50-238, 51-238, 52-238, 53-238, 54-238, 55-238, 56-238, 57-238, 58-238, 59-238, 60-238, 61-238, 62-238, 63-238, 64-238, 65-238, 66-238, 67-238, 68-238, 69-238, 70-238, 71-238, 72-238, 73-238, 74-238, 75-238, 76-238, 77-238, 78-238, 79-238, 80-238, 81-238, 82-238, 83-238, 84-238, 85-238, 86-238, 87-238, 88-238, 89-238, 90-238, 91-238, 92-238, 93-238, 94-238, 95-238, 96-238, 97-238, 98-238, 99-238, 100-238, 101-238, 102-238, 103-238, 104-238, 105-238, 106-238, 107-238, 108-238, 109-238 or 110-238 of SEQ ID NO: 6, where it is understood that amino acid 16 is taken to represent the N-terminal amino acid of mature XCRF4 polypeptide absent the signal peptide.

In more preferred embodiments, XCRF4 polypeptide fragments having activity are selected from amino acids 16-238, 18-238, 28-238, 30-238, 39-238, 58-238, 63-238, 64-238, 66-238, 70-238, 79-238, 87-238, 97-238, 98-238, 99-238, 100-238, 101-238, 102-238, 103-238, 104-238, 105-238, 106-238,

107-238, 108-238, 109-238 or 110-238 of SEQ ID NO: 6, where it is understood that amino acid 16 is taken to represent the N-terminal amino acid of mature XCRF4 polypeptide absent the signal peptide.

In yet more preferred embodiments, XCRF4 polypeptide fragments having activity are selected from amino acids 16-238, 97-238, 98-238, 99-238, 100-238, 101-238, 102-238, 103-238, 104-238, 105-238, 106-238, 107-238, 108-238, 109-238 or 110-238 of SEQ ID NO: 6, where it is understood that amino acid 16 is taken to represent the N-terminal amino acid of mature XCRF4 polypeptide absent the signal peptide.

In further preferred embodiments, said polypeptide fragment comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the corresponding consecutive amino acids of the polypeptide sequences identified in SEQ ID NO: 6.

The invention further provides purified, isolated, or recombinant XCRF4 polypeptides that have weight reduction activities. Preferred XCRF4 polypeptide fragments are said polypeptide fragments having activity, wherein said activity is also selected from the group consisting of prevention of weight gain, weight reduction, and maintenance of weight loss. In preferred embodiments, said polypeptide fragment comprises, consists essentially of, or consists of, at least 6 consecutive amino acids and not more than 238 consecutive amino acids of SEQ ID NO: 6.

In other preferred embodiments, XCRF4 polypeptide fragments having activity are selected from amino acids 16-238, 17-238, 18-238, 19-238, 20-238, 21-238, 22-238, 23-238, 24-238, 25-238, 26-238, 27-238, 28-238, 29-238, 30-238, 31-238, 32-238, 33-238, 34-238, 35-238, 36-238, 37-238, 38-238, 39-238, 40-238, 41-238, 42-238, 43-238, 44-238, 45-238, 46-238, 47-238, 48-238, 49-238, 50-238, 51-238, 52-238, 53-238, 54-238, 55-238, 56-238, 57-238, 58-238, 59-238, 60-238, 61-238, 62-238, 63-238, 64-238, 65-238, 66-238, 67-238, 68-238, 69-238, 70-238, 71-238, 72-238, 73-238, 74-238, 75-238, 76-238, 77-238, 78-238, 79-238, 80-238, 81-238, 82-238, 83-238, 84-238, 85-238, 86-238, 87-238, 88-238, 89-238, 90-238, 91-238, 92-238, 93-238, 94-238, 95-238, 96-238, 97-238, 98-238, 99-238, 100-238, 101-238, 102-238, 103-238, 104-238, 105-238, 106-238, 107-238, 108-238, 109-238 or 110-238 of SEQ ID NO: 6, where it is understood that amino acid 16 is taken to represent the N-terminal amino acid of mature XCRF4 polypeptide absent the signal peptide.

In more preferred embodiments, XCRF4 polypeptide fragments having activity are selected from amino acids 16-238, 18-238, 28-238, 30-238, 39-238, 58-238, 63-238, 64-238, 66-238, 70-238, 79-238, 87-238, 97-238, 98-238, 99-238, 100-238, 101-238, 102-238, 103-238, 104-238, 105-238, 106-238, 107-238, 108-238, 109-238 or 110-238 of SEQ ID NO: 6, where it is understood that amino acid 16 is taken to represent the N-terminal amino acid of mature XCRF4 polypeptide absent the signal peptide.

In yet more preferred embodiments, XCRF4 polypeptide fragments having activity are selected from amino acids 16-238, 97-238, 98-238, 99-238, 100-238, 101-238, 102-238, 103-238, 104-238, 105-

238, 106-238, 107-238, 108-238, 109-238 or 110-238 of SEQ ID NO: 6, where it is understood that amino acid 16 is taken to represent the N-terminal amino acid of mature XCRF4 polypeptide absent the signal peptide.

In further preferred embodiments, said polypeptide fragment comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the corresponding consecutive amino acids of the polypeptide sequences identified in SEQ ID NO: 6.

The invention yet further provides a purified or isolated polypeptide comprising, consisting of, or consisting essentially of an amino acid sequence selected from the group consisting of: (a) a full-length XCRF4 polypeptide at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the corresponding polypeptide of SEQ ID NO: 6; (b) a full-length XCRF4 polypeptide of SEQ ID NO: 6 absent the N-terminal Met; (c) a mature XCRF4 polypeptide of SEQ ID NO: 2 lacking signal peptide; (d) an XCRF4 polypeptide of SEQ ID NO: 6 wherein said XCRF4 polypeptide is of any one integer in length between 6 amino acids and 238 amino acids (full-length) inclusive of SEQ ID NO: 2; (e) the epitope-bearing fragments of an XCRF4 polypeptide of SEQ ID NO: 6; (f) the allelic variant polypeptides of any of the polypeptides of (a)-(e). The invention further provides for fragments of the polypeptides of (a)-(f) above, such as those having biological activity or comprising biologically functional domain(s).

In other highly preferred embodiments, XCRF4 polypeptides comprise, consist essentially of, or consist of, a purified, isolated, or a recombinant XCRF4 polypeptide fragment comprised of all or part of the globular C-terminal C1q homology domain. Preferably, said XCRF4 polypeptide fragment comprises, consists essentially of, or consists of, at least 6 consecutive amino acids of amino acids 16-238 of SEQ ID NO: 6, where it is understood that amino acid 16 is taken to represent the N-terminal amino acid of mature XCRF4 polypeptide absent the signal peptide. In other preferred embodiments, said XCRF4 polypeptide fragments comprising all or part of the globular C-terminal C1q homology domain are selected from amino acids 16-238, 17-238, 18-238, 19-238, 20-238, 21-238, 22-238, 23-238, 24-238, 25-238, 26-238, 27-238, 28-238, 29-238, 30-238, 31-238, 32-238, 33-238, 34-238, 35-238, 36-238, 37-238, 38-238, 39-238, 40-238, 41-238, 42-238, 43-238, 44-238, 45-238, 46-238, 47-238, 48-238, 49-238, 50-238, 51-238, 52-238, 53-238, 54-238, 55-238, 56-238, 57-238, 58-238, 59-238, 60-238, 61-238, 62-238, 63-238, 64-238, 65-238, 66-238, 67-238, 68-238, 69-238, 70-238, 71-238, 72-238, 73-238, 74-238, 75-238, 76-238, 77-238, 78-238, 79-238, 80-238, 81-238, 82-238, 83-238, 84-238, 85-238, 86-238, 87-238, 88-238, 89-238, 90-238, 91-238, 92-238, 93-238, 94-238, 95-238, 96-238, 97-238, 98-238, 99-238, 100-238, 101-238, 102-238, 103-238, 104-238, 105-238, 106-238, 107-238, 108-238, 109-238 or 110-238 of SEQ ID NO: 6, where it is understood that amino acid 16 is taken to represent the N-terminal amino acid of mature XCRF4 polypeptide absent the signal peptide.

In more preferred embodiments, XCRF4 polypeptide fragments comprising all or part of the globular C-terminal C1q homology domain are selected from amino acids 16-238, 18-238, 28-238, 30-238, 39-238, 58-238, 63-238, 64-238, 66-238, 70-238, 79-238, 87-238, 97-238, 98-238, 99-238, 100-238, 101-238, 102-238, 103-238, 104-238, 105-238, 106-238, 107-238, 108-238, 109-238 or 110-238 of SEQ ID NO: 6, where it is understood that amino acid 16 is taken to represent the N-terminal amino acid of mature XCRF4 polypeptide absent the signal peptide.

In yet more preferred embodiments, XCRF4 polypeptide fragments comprising all or part of the globular C-terminal C1q homology domain are selected from amino acids 16-238, 97-238, 98-238, 99-238, 100-238, 101-238, 102-238, 103-238, 104-238, 105-238, 106-238, 107-238, 108-238, 109-238 or 110-238 of SEQ ID NO: 6, where it is understood that amino acid 16 is taken to represent the N-terminal amino acid of mature XCRF4 polypeptide absent the signal peptide.

Alternatively, said XCRF4 polypeptide fragment comprises, consists essentially of, or consists of, an amino acid sequence at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the corresponding amino acids 97-238 of SEQ ID NO: 6.

In a further aspect, the invention features purified, isolated, or recombinant XCRF5 polypeptides that have lipid partitioning, lipid metabolism, and insulin-like activities. Preferred XCRF5 polypeptide fragments are said polypeptide fragments having activity, wherein said activity is also selected from the group consisting of lipid partitioning, lipid metabolism, and insulin-like activity. In preferred embodiments, said polypeptide fragment comprises, consists essentially of, or consists of, at least 6 consecutive amino acids and not more than 287 consecutive amino acids of SEQ ID NO: 8.

In other preferred embodiments, XCRF5 polypeptide fragments having activity are selected from amino acids 22-287, 23-287, 24-287, 25-287, 26-287, 27-287, 28-287, 29-287, 30-287, 31-287, 32-287, 33-287, 34-287, 35-287, 36-287, 37-287, 38-287, 39-287, 40-287, 41-287, 42-287, 43-287, 44-287, 45-287, 46-287, 47-287, 48-287, 49-287, 50-287, 51-287, 52-287, 53-287, 54-287, 55-287, 56-287, 57-287, 58-287, 59-287, 60-287, 61-287, 62-287, 63-287, 64-287, 65-287, 66-287, 67-287, 68-287, 69-287, 70-287, 71-287, 72-287, 73-287, 74-287, 75-287, 76-287, 77-287, 78-287, 79-287, 80-287, 81-287, 82-287, 83-287, 84-287, 85-287, 86-287, 87-287, 88-287, 89-287, 90-287, 91-287, 92-287, 93-287, 94-287, 95-287, 96-287, 97-287, 98-287, 99-287, 100-287, 101-287, 102-287, 103-287, 104-287, 105-287, 106-287, 107-287, 108-287, 109-287, 110-287, 111-287, 112-287, 113-287, 114-287, 115-287, 116-287, 117-287, 118-287, 119-287, 120-287, 121-287, 122-287, 123-287, 124-287, 125-287, 126-287, 127-287, 128-287, 129-287, 130-287, 131-287, 132-287, 133-287, 134-287, 135-287, 136-287, 137-287, 138-287, 139-287, 140-287, 141-287, 142-287, 143-287, 144-287, 145-287, 146-287, 147-287, 148-287, 149-287, 150-287, 151-287, 152-287, 153-287, 154-287, 155-287, 156-287, 157-287, 158-287 or 159-287 of SEQ

ID NO: 8, where it is understood that amino acid 22 is taken to represent the N-terminal amino acid of mature XCRF5 polypeptide absent the signal peptide.

In more preferred embodiments, XCRF5 polypeptide fragments having activity are selected from amino acids 22-287, 121-287, 122-287, 123-287, 124-287, 125-287, 126-287, 127-287, 128-287, 129-287, 130-287, 131-287, 132-287, 133-287, 134-287, 135-287, 136-287, 137-287, 138-287, 139-287, 140-287, 141-287, 142-287, 143-287, 144-287, 145-287, 146-287, 147-287, 148-287, 149-287, 150-287, 151-287, 152-287, 153-287, 154-287, 155-287, 156-287, 157-287, 158-287 or 159-287 of SEQ ID NO: 8, where it is understood that amino acid 22 is taken to represent the N-terminal amino acid of mature XCRF5 polypeptide absent the signal peptide.

In yet more preferred embodiments, XCRF5 polypeptide fragments having activity are selected from amino acids 22-287, 123-287, 124-287, 125-287, 126-287, 127-287, 128-287, 129-287, 130-287, 131-287, 132-287, 133-287, 134-287, 135-287, 136-287, 137-287, 138-287, 139-287, 140-287, 141-287, 142-287, 143-287, 144-287, 145-287, 146-287, 147-287, 148-287, 149-287, 150-287, 151-287, 152-287 or 153-287 of SEQ ID NO: 82, where it is understood that amino acid 22 is taken to represent the N-terminal amino acid of mature XCRF5 polypeptide absent the signal peptide.

In further preferred embodiments, said polypeptide fragment comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the corresponding consecutive amino acids of the polypeptide sequences identified in SEQ ID NO: 8. In yet further preferred embodiments, said polypeptide fragment comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the corresponding consecutive amino acids of the polypeptide sequences identified in SEQ ID NO: 8, wherein said amino acid sequence comprises any one of or any combination of alanine at position 123, glycine at position 124, threonine at position 125, serine at position 127, glycine at position 133, alanine at position 136, valine at position 138, serine at position 142, or threonine at position 153 of SEQ ID NO: 8.

The invention further provides purified, isolated, or recombinant XCRF5 polypeptides that have weight reduction activities. Preferred XCRF5 polypeptide fragments are said polypeptide fragments having activity, wherein said activity is also selected from the group consisting of prevention of weight gain, weight reduction, and maintenance of weight loss. In preferred embodiments, said polypeptide fragment comprises, consists essentially of, or consists of, at least 6 consecutive amino acids and not more than 287 consecutive amino acids of SEQ ID NO: 8.

In other preferred embodiments, XCRF5 polypeptide fragments having activity are selected from amino acids 22-287, 23-287, 24-287, 25-287, 26-287, 27-287, 28-287, 29-287, 30-287, 31-287, 32-287, 33-287, 34-287, 35-287, 36-287, 37-287, 38-287, 39-287, 40-287, 41-287, 42-287, 43-287, 44-287,

45-287, 46-287, 47-287, 48-287, 49-287, 50-287, 51-287, 52-287, 53-287, 54-287, 55-287, 56-287, 57-287, 58-287, 59-287, 60-287, 61-287, 62-287, 63-287, 64-287, 65-287, 66-287, 67-287, 68-287, 69-287, 70-287, 71-287, 72-287, 73-287, 74-287, 75-287, 76-287, 77-287, 78-287, 79-287, 80-287, 81-287, 82-287, 83-287, 84-287, 85-287, 86-287, 87-287, 88-287, 89-287, 90-287, 91-287, 92-287, 93-287, 94-287, 95-287, 96-287, 97-287, 98-287, 99-287, 100-287, 101-287, 102-287, 103-287, 104-287, 105-287, 106-287, 107-287, 108-287, 109-287, 110-287, 111-287, 112-287, 113-287, 114-287, 115-287, 116-287, 117-287, 118-287, 119-287, 120-287, 121-287, 122-287, 123-287, 124-287, 125-287, 126-287, 127-287, 128-287, 129-287, 130-287, 131-287, 132-287, 133-287, 134-287, 135-287, 136-287, 137-287, 138-287, 139-287, 140-287, 141-287, 142-287, 143-287, 144-287, 145-287, 146-287, 147-287, 148-287, 149-287, 150-287, 151-287, 152-287, 153-287, 154-287, 155-287, 156-287, 157-287, 158-287 or 159-287 of SEQ ID NO: 8, where it is understood that amino acid 22 is taken to represent the N-terminal amino acid of mature XCRF5 polypeptide absent the signal peptide.

In more preferred embodiments, XCRF5 polypeptide fragments having activity are selected from amino acids 22-287, 121-287, 122-287, 123-287, 124-287, 125-287, 126-287, 127-287, 128-287, 129-287, 130-287, 131-287, 132-287, 133-287, 134-287, 135-287, 136-287, 137-287, 138-287, 139-287, 140-287, 141-287, 142-287, 143-287, 144-287, 145-287, 146-287, 147-287, 148-287, 149-287, 150-287, 151-287, 152-287, 153-287, 154-287, 155-287, 156-287, 157-287, 158-287 or 159-287 of SEQ ID NO: 8, where it is understood that amino acid 22 is taken to represent the N-terminal amino acid of mature XCRF5 polypeptide absent the signal peptide.

In yet more preferred embodiments, XCRF5 polypeptide fragments having activity are selected from amino acids 22-287, 123-287, 124-287, 125-287, 126-287, 127-287, 128-287, 129-287, 130-287, 131-287, 132-287, 133-287, 134-287, 135-287, 136-287, 137-287, 138-287, 139-287, 140-287, 141-287, 142-287, 143-287, 144-287, 145-287, 146-287, 147-287, 148-287, 149-287, 150-287, 151-287, 152-287 or 153-287 of SEQ ID NO: 8, where it is understood that amino acid 22 is taken to represent the N-terminal amino acid of mature XCRF5 polypeptide absent the signal peptide.

In further preferred embodiments, said polypeptide fragment comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the corresponding consecutive amino acids of the polypeptide sequences identified in SEQ ID NO: 8. In yet further preferred embodiments, said polypeptide fragment comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the corresponding consecutive amino acids of the polypeptide sequences identified in SEQ ID NO: 8, wherein said amino acid sequence comprises any one of or any combination of alanine at position 123, glycine at position 124, threonine at position 125, serine at position 127, glycine at position 133, alanine

at position 136, valine at position 138, serine at position 142, or threonine at position 153 of SEQ ID NO: 8.

The invention yet further provides a purified or isolated polypeptide comprising, consisting of, or consisting essentially of an amino acid sequence selected from the group consisting of: (a) a full-length XCRF5 polypeptide at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the corresponding polypeptide of SEQ ID NO: 8; (b) a full-length XCRF5 polypeptide at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the corresponding polypeptide of SEQ ID NO: 8, wherein said full-length XCRF5 polypeptide comprises any one of or any combination of alanine at position 123, glycine at position 124, threonine at position 125, serine at position 127, glycine at position 133, alanine at position 136, valine at position 138, serine at position 142, or threonine at position 153 of SEQ ID NO: 8; (c) a full-length XCRF5 polypeptide of SEQ ID NO: 8 absent the N-terminal Met; (d) a mature XCRF5 polypeptide of SEQ ID NO: 2 lacking signal peptide; (e) an XCRF5 polypeptide of SEQ ID NO: 8 wherein said XCRF5 polypeptide is of any one integer in length between 6 amino acids and 287 amino acids (full-length) inclusive of SEQ ID NO: 8; (f) the epitope-bearing fragments of an XCRF5 polypeptide of SEQ ID NO: 8; (g) the allelic variant polypeptides of any of the polypeptides of (a)-(f). The invention further provides for fragments of the polypeptides of (a)-(g) above, such as those having biological activity or comprising biologically functional domain(s).

In other highly preferred embodiments, XCRF5 polypeptides comprise, consist essentially of, or consist of, a purified, isolated, or a recombinant XCRF5 polypeptide fragment comprised of all or part of the globular C-terminal C1q homology domain. Preferably, said XCRF5 polypeptide fragment comprises, consists essentially of, or consists of, at least 6 consecutive amino acids of amino acids 22-287 of SEQ ID NO: 8, where it is understood that amino acid 22 is taken to represent the N-terminal amino acid of mature XCRF5 polypeptide absent the signal peptide. In other preferred embodiments, said XCRF5 polypeptide fragments comprising all or part of the globular C-terminal C1q homology domain are selected from amino acids 22-287, 23-287, 24-287, 25-287, 26-287, 27-287, 28-287, 29-287, 30-287, 31-287, 32-287, 33-287, 34-287, 35-287, 36-287, 37-287, 38-287, 39-287, 40-287, 41-287, 42-287, 43-287, 44-287, 45-287, 46-287, 47-287, 48-287, 49-287, 50-287, 51-287, 52-287, 53-287, 54-287, 55-287, 56-287, 57-287, 58-287, 59-287, 60-287, 61-287, 62-287, 63-287, 64-287, 65-287, 66-287, 67-287, 68-287, 69-287, 70-287, 71-287, 72-287, 73-287, 74-287, 75-287, 76-287, 77-287, 78-287, 79-287, 80-287, 81-287, 82-287, 83-287, 84-287, 85-287, 86-287, 87-287, 88-287, 89-287, 90-287, 91-287, 92-287, 93-287, 94-287, 95-287, 96-287, 97-287, 98-287, 99-287, 100-287, 101-287, 102-287, 103-287, 104-287, 105-287, 106-287, 107-287, 108-287, 109-287, 110-287, 111-287, 112-287, 113-287, 114-287, 115-287, 116-287, 117-287, 118-287, 119-287, 120-287, 121-287, 122-287, 123-287, 124-287, 125-287, 126-287, 127-287, 128-287, 129-287, 130-287, 131-287, 132-287, 133-287, 134-287, 135-287,

136-287, 137-287, 138-287, 139-287, 140-287, 141-287, 142-287, 143-287, 144-287, 145-287, 146-287, 147-287, 148-287, 149-287, 150-287, 151-287, 152-287, 153-287, 154-287, 155-287, 156-287, 157-287, 158-287 or 159-287 of SEQ ID NO: 8, where it is understood that amino acid 22 is taken to represent the N-terminal amino acid of mature XCRF5 polypeptide absent the signal peptide.

In more preferred embodiments, XCRF5 polypeptide fragments comprising all or part of the globular C-terminal C1q homology domain are selected from amino acids 22-287, 121-287, 122-287, 123-287, 124-287, 125-287, 126-287, 127-287, 128-287, 129-287, 130-287, 131-287, 132-287, 133-287, 134-287, 135-287, 136-287, 137-287, 138-287, 139-287, 140-287, 141-287, 142-287, 143-287, 144-287, 145-287, 146-287, 147-287, 148-287, 149-287, 150-287, 151-287, 152-287, 153-287, 154-287, 155-287, 156-287, 157-287, 158-287 or 159-287 of SEQ ID NO: 8, where it is understood that amino acid 22 is taken to represent the N-terminal amino acid of mature XCRF5 polypeptide absent the signal peptide.

In yet more preferred embodiments, XCRF5 polypeptide fragments comprising all or part of the globular C-terminal C1q homology domain are selected from amino acids 22-287, 123-287, 124-287, 125-287, 126-287, 127-287, 128-287, 129-287, 130-287, 131-287, 132-287, 133-287, 134-287, 135-287, 136-287, 137-287, 138-287, 139-287, 140-287, 141-287, 142-287, 143-287, 144-287, 145-287, 146-287, 147-287, 148-287, 149-287, 150-287, 151-287, 152-287 or 153-287 of SEQ ID NO: 8, where it is understood that amino acid 22 is taken to represent the N-terminal amino acid of mature XCRF5 polypeptide absent the signal peptide.

Alternatively, said XCRF5 polypeptide fragment comprises, consists essentially of, or consists of, an amino acid sequence at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the corresponding amino acids 121-287 of SEQ ID NO: 8. Further alternatively, said XCRF5 polypeptide fragment more preferably comprises, consists essentially of, or consists of, an amino acid sequence at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the corresponding amino acids 121-287 of SEQ ID NO: 8, wherein said amino acid sequence comprises any one of or any combination of alanine at position 123, glycine at position 124, threonine at position 125, serine at position 127, glycine at position 133, alanine at position 136, valine at position 138, serine at position 142, or threonine at position 153 of SEQ ID NO: 8.

In a further preferred embodiment, XCRF polypeptides are able to lower circulating (either in blood, serum or plasma) levels (concentration) of: (i) free fatty acids, (ii) glucose, and/or (iii) triglycerides. Further preferred polypeptides of the invention demonstrating free fatty acid level lowering activity, glucose level lowering activity, and/or triglyceride level lowering activity, have an activity that is the same or greater than full-length XCRF polypeptides at the same molar concentration, have the same or greater than transient activity and/or have a sustained activity.

In other further preferred embodiment, XCRF polypeptides are able to prevent weight gain, reduce weight, and/or maintain weight loss. Further preferred polypeptides of the invention demonstrating prevention of weight gain, weight reduction, and/or maintenance of weight loss, have an activity that is the same or greater than full-length XCRF polypeptides at the same molar concentration, have the same or greater than transient activity and/or have a sustained activity.

Further preferred XCRF polypeptides are those that maintain weight loss, preferably in individuals who were previously "obese" and are now "healthy" (as defined herein).

Further preferred XCRF polypeptides are those that significantly stimulate muscle lipid or free fatty acid oxidation. Further preferred XCRF polypeptides are those that significantly stimulate muscle lipid or free fatty acid oxidation.

Further preferred XCRF polypeptides are those that cause C2C12 cells differentiated in the presence of said polypeptides to undergo at least 10%, 20%, 30%, 35%, or 40% more oleate oxidation as compared to untreated cells.

Further preferred XCRF polypeptides are those that increase leptin uptake in a liver cell line (preferably BPRCL mouse liver cells (ATCC CRL-2217)).

Further preferred XCRF polypeptides are those that significantly reduce the postprandial increase in plasma free fatty acids due to a high fat meal.

Further preferred XCRF polypeptides are those that significantly reduce or eliminate ketone body production as the result of a high fat meal.

Further preferred XCRF polypeptides are those that increase glucose uptake in skeletal muscle cells.

Further preferred XCRF polypeptides are those that increase glucose uptake in adipose cells.

Further preferred XCRF polypeptides are those that increase glucose uptake in neuronal cells.

Further preferred XCRF polypeptides are those that increase glucose uptake in red blood cells.

Further preferred XCRF polypeptides are those that increase glucose uptake in the brain.

Further preferred XCRF polypeptides are those that significantly reduce the postprandial increase in plasma glucose following a meal, particularly a high carbohydrate meal.

Further preferred XCRF polypeptides are those that significantly prevent the postprandial increase in plasma glucose following a meal, particularly a high fat or a high carbohydrate meal.

Further preferred XCRF polypeptides are those that increase insulin sensitivity.

Further preferred XCRF polypeptides are those that inhibit the progression from impaired glucose tolerance to insulin resistance.

Further preferred XCRF polypeptides are those that form multimers (e.g., heteromultimers or homomultimers) *in vitro* and/or *in vivo*. Preferred multimers are homotrimers or homohexamers. Other

preferred multimers are homomultimers comprising at least 2, 3, 4, 6, 8, 9, 10 or 12 XCRF polypeptide subunits. Further preferred multimers are heterotrimers or heterohexamers. Other preferred multimers are heteromultimers comprising at least 1, 2, 3, 4, 6, 8, 9, 10 or 12 XCRF polypeptide subunits.

Further preferred embodiments include heterologous polypeptides comprising one of the XCRF polypeptides of the invention. More preferred is said heterologous polypeptide comprised of a signal peptide fused to the N-terminus of said XCRF polypeptide of the invention. In yet more preferred embodiment, said signal peptide is human zinc-alpha 2-glycoprotein signal peptide of amino acid sequence MVRMVPVLLSLLLLLGPAVP, preferably encoded by the polynucleotide of sequence atggtaagaatggtgcctgtcctgctgtctctgctgctgcttctgggtcctgctgtcccc.

In a second aspect, the invention features purified, isolated, or recombinant polynucleotides encoding said XCRF polypeptides described in the first aspect, or the complement thereof. In further embodiments the polynucleotides are DNA, RNA, DNA/RNA hybrids, single-stranded, and double-stranded.

In a third aspect, the invention features a recombinant vector comprising, consisting essentially of, or consisting of, said polynucleotide described in the second aspect.

In a fourth aspect, the invention features a recombinant cell comprising, consisting essentially of, or consisting of said recombinant vector described in the third aspect. A further embodiment includes a host cell recombinant for a polynucleotide of the invention.

In a fifth aspect, the invention features a pharmaceutical or physiologically acceptable composition comprising, consisting essentially of, or consisting of, said XCRF polypeptides described in the first aspect and, alternatively, a pharmaceutical or physiologically acceptable diluent.

In a sixth aspect, the invention features a method of reducing body mass comprising providing or administering to individuals in need of reducing body mass said pharmaceutical or physiologically acceptable composition described in the fifth aspect.

In a further preferred embodiment, the invention features a method of complementary therapy of reducing body mass comprising providing or administering to individuals in need of reducing body mass said pharmaceutical or physiologically acceptable composition described in the fifth aspect in combination with a weight reducing agent. Examples of said weight reducing agent include lipase inhibitors, such as orlistat, and serotonin reuptake inhibitors (SSRI) and noradrenaline reuptake inhibitor, such as sibutramine.

In further preferred embodiments, the invention features a method of maintaining a reduced body mass comprising providing or administering to individuals in need of maintaining a reduced body mass said pharmaceutical or physiologically acceptable composition described in the fifth aspect. Further preferred is a method of maintaining a reduced body fat mass that comprises providing or

administering to individuals in need thereof said pharmaceutical or physiologically acceptable composition described in the fifth aspect, returning energy intake to a normal level in said individual, and maintaining increased energy expenditure in said individual. Preferably, said individual is able to maintain a stable weight that is 10-20% below said individual's obese weight (as defined herein). In preferred embodiments, said individual is a mammal, preferably a human.

In other preferred embodiments, the invention features a method of maintaining weight loss comprising providing or administering to individuals in need of maintaining weight loss said pharmaceutical or physiologically acceptable composition described in the fifth aspect in combination with reduced energy intake and/or increased energy expenditure.

In preferred embodiments, the identification of said individuals in need of reducing body mass to be treated with said pharmaceutical or physiologically acceptable composition comprises genotyping XCRF single nucleotide polymorphisms (SNPs) or measuring XCRF polypeptide or mRNA levels in clinical samples from said individuals. Preferably, said clinical samples are selected from the group consisting of plasma, urine, and saliva. Preferably, an XCRF polypeptide fragment of the present invention is administered to an individual with at least a 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% reduction in blood, serum or plasma levels of full-length any one or all of the XCRF polypeptides or the naturally proteolytically cleaved XCRF fragments as compared to healthy, non-obese patients.

In a seventh aspect, the invention features a method of preventing or treating an metabolic-related disease or disorder comprising providing or administering to an individual in need of such treatment said pharmaceutical or physiologically acceptable composition described in the fifth aspect. In preferred embodiments, the identification of said individuals in need of such treatment to be treated with said pharmaceutical or physiologically acceptable composition comprises genotyping XCRF single nucleotide polymorphisms (SNPs) or measuring XCRF polypeptide or mRNA levels in clinical samples from said individuals. Preferably, said clinical samples are selected from the group consisting of blood, serum, plasma, urine, and saliva. Preferably, said metabolic-related disease or disorder is selected from the group consisting of obesity, impaired glucose tolerance, insulin resistance, atherosclerosis, atheromatous disease, heart disease, hypertension, stroke, Syndrome X, non-insulin-dependent diabetes and Type II diabetes. Type II diabetes-related complications to be treated by the methods of the invention include microangiopathic lesions, ocular lesions, and renal lesions. Heart disease includes, but is not limited to, cardiac insufficiency, coronary insufficiency, and high blood pressure. Other metabolic-related disorders to be treated by compounds of the invention include hyperlipidemia and hyperuricemia. Yet other metabolic-related diseases or disorders of the invention include cachexia,

wasting, AIDS-related weight loss, cancer-related weight loss, anorexia, and bulimia. In preferred embodiments, said individual is a mammal, preferably a human.

In related aspects, embodiments of the present invention includes methods of causing or inducing a desired biological response in an individual comprising the steps of: providing or administering to an individual a composition comprising an XCRF polypeptide, wherein said biological response is selected from the group consisting of:

(a) modulating circulating (either blood, serum, or plasma) levels (concentration) of free fatty acids, wherein said modulating is preferably lowering;

(b) modulating circulating (either blood, serum or plasma) levels (concentration) of glucose, wherein said modulating is preferably lowering;

(c) modulating circulating (either blood, serum or plasma) levels (concentration) of triglycerides, wherein said modulating is preferably lowering;

(d) stimulating muscle lipid or free fatty acid oxidation;

(c) modulating leptin uptake in the liver or liver cells, wherein said modulating is preferably increasing;

(e) modulating the postprandial increase in plasma free fatty acids due to a high fat meal, wherein said modulating is preferably reducing;

(f) modulating ketone body production as the result of a high fat meal, wherein said modulating is preferably reducing or eliminating;

(g) increasing cell or tissue sensitivity to insulin, particularly muscle, adipose, liver or brain; and

(h) inhibiting the progression from impaired glucose tolerance to insulin resistance;

and further wherein said biological response is significantly greater than, or at least 10%, 20%, 30% , 35%, 40%, 50% 75% 100% or 500% greater than, the biological response caused or induced by insulin alone at the same molar concentration. In further preferred embodiments, the present invention of said pharmaceutical or physiologically acceptable composition can be used as a method to control blood glucose in some persons with Non-Insulin Dependent Diabetes Mellitus (NIDDM, Type II diabetes) in combination with insulin therapy.

In further preferred embodiments, the present invention of said pharmaceutical or physiologically acceptable composition can be used as a method to control blood glucose in some persons with Insulin Dependent Diabetes Mellitus (IDDM, Type I diabetes) in combination with insulin therapy.

In further preferred embodiments, the present invention of said pharmaceutical or physiologically acceptable composition can be used as a method to control body weight in some persons

with Non-Insulin Dependent Diabetes Mellitus (NIDDM, Type II diabetes) in combination with insulin therapy.

In further preferred embodiments, the present invention of said pharmaceutical or physiologically acceptable composition can be used as a method to control body weight in some persons with Insulin Dependent Diabetes Mellitus (IDDM, Type I diabetes) in combination with insulin therapy.

In further preferred embodiments, the present invention of said pharmaceutical or physiologically acceptable composition can be used as a method to control blood glucose in some persons with Non-Insulin Dependent Diabetes Mellitus (NIDDM, Type II diabetes) alone, without combination of insulin therapy.

In further preferred embodiments, the present invention of said pharmaceutical or physiologically acceptable composition can be used as a method to control blood glucose in some persons with Insulin Dependent Diabetes Mellitus (IDDM, Type I diabetes) alone, without combination of insulin therapy.

In further preferred embodiments, the present invention of said pharmaceutical or physiologically acceptable composition can be used as a method to control body weight in some persons with Non-Insulin Dependent Diabetes Mellitus (NIDDM, Type II diabetes) alone, without combination of insulin therapy.

In further preferred embodiments, the present invention of said pharmaceutical or physiologically acceptable composition can be used as a method to control body weight in some persons with Insulin Dependent Diabetes Mellitus (IDDM, Type I diabetes) alone, without combination of insulin therapy.

In a further preferred embodiment, the present invention may be used in complementary therapy of NIDDM patients to improve their weight or glucose control in combination with an insulin secretagogue (preferably oral form) or an insulin sensitising (preferably oral form) agent. Preferably, the oral insulin secretagogue is 1,1-dimethyl-2-(2-morpholino phenyl)guanidine fumarate (BTS67582) or a sulphonylurea selected from tolbutamide, tolazamide, chlorpropamide, glibenclamide, glimepiride, glipizide and glidazide. Preferably, the insulin sensitising agent is selected from metformin, ciglitazone, troglitazone and pioglitazone.

The present invention further provides a method of improving the body weight or glucose control of NIDDM patients alone, without an insulin secretagogue or an insulin sensitising agent.

In a further preferred embodiment, the present invention may be used in complementary therapy of IDDM patients to improve their weight or glucose control in combination with an insulin secretagogue (preferably oral form) or an insulin sensitising (preferably oral form) agent. Preferably, ~~the~~ insulin secretagogue is 1,1-dimethyl-2-(2-morpholino phenyl)guanidine fumarate (BTS67582) or a

sulphonylurea selected from tolbutamide, tolazamide, chlorpropamide, glibenclamide, glimepiride, glipizide and glidazide. Preferably, the insulin sensitising agent is selected from metformin, ciglitazone, troglitazone and pioglitazone.

The present invention further provides a method of improving the body weight or glucose control of IDDM patients alone, without an insulin secretagogue or an insulin sensitising agent.

In a further preferred embodiment, the present invention may be administered either concomitantly or concurrently, with the insulin secretagogue or insulin sensitising agent for example in the form of separate dosage units to be used simultaneously, separately or sequentially (either before or after the secretagogue or either before or after the sensitising agent). Accordingly, the present invention further provides for a composition of pharmaceutical or physiologically acceptable composition and an insulin secretagogue or insulin sensitising agent as a combined preparation for simultaneous, separate or sequential use for the improvement of body weight or glucose control in NIDDM or IDDM patients.

In further preferred embodiments, the present invention of said pharmaceutical or physiologically acceptable composition further provides a method for the use as an insulin sensitiser.

In further preferred embodiments, the present invention of said pharmaceutical or physiologically acceptable composition can be used as a method to improve insulin sensitivity in some persons with Non-Insulin Dependent Diabetes Mellitus (NIDDM, Type II diabetes) in combination with insulin therapy.

In further preferred embodiments, the present invention of said pharmaceutical or physiologically acceptable composition can be used as a method to improve insulin sensitivity in some persons with Insulin Dependent Diabetes Mellitus (IDDM, Type I diabetes) in combination with insulin therapy.

In further preferred embodiments, the present invention of said pharmaceutical or physiologically acceptable composition can be used as a method to improve insulin sensitivity in some persons with Non-Insulin Dependent Diabetes Mellitus (NIDDM, Type II diabetes) without insulin therapy.

In an eighth aspect, the invention features a method of making the XCRF polypeptides described in the first aspect, wherein said method is selected from the group consisting of: proteolytic cleavage, recombinant methodology and artificial synthesis.

In a ninth aspect, the present invention provides a method of making a recombinant XCRF polypeptide fragment or a full-length XCRF polypeptide, the method comprising providing a transgenic, non-human mammal whose milk contains said recombinant XCRF polypeptide fragment or full-length protein, and purifying said recombinant XCRF polypeptide fragment or said full-length XCRF polypeptide from the milk of said non-human mammal. In one embodiment, said non-human mammal

is a cow, goat, sheep, rabbit, or mouse. In another embodiment, the method comprises purifying a recombinant mature XCRF polypeptide absent the signal peptide from said milk, and further comprises cleaving said protein *in vitro* to obtain a desired XCRF polypeptide fragment.

In a tenth aspect, the invention features a use of the polypeptide described in the first aspect for the preparation of a medicament for the treatment of obesity-related diseases and disorders and/or for reducing body mass. Preferably, said metabolic-related diseases and disorders are selected from the group consisting of obesity, insulin resistance, atherosclerosis, atheromatous disease, heart disease, hypertension, stroke, Syndrome X, non-insulin-dependent diabetes and Type II diabetes. Type II diabetes-related complications to be treated by the methods of the invention include microangiopathic lesions, ocular lesions, and renal lesions. Heart disease includes, but is not limited to, cardiac insufficiency, coronary insufficiency, and high blood pressure. Other metabolic-related disorders to be treated by compounds of the invention include hyperlipidemia and hyperuricemia. Yet other metabolic-related diseases or disorders of the invention include cachexia, wasting, AIDS-related weight loss, anorexia, and bulimia. In preferred embodiments, said individual is a mammal, preferably a human.

The invention further features a use of the polypeptide described in the first aspect for the preparation of a medicament for prevention of weight gain, for weight reduction, and/or for maintenance of weight loss. In preferred embodiments, said individual is a mammal, preferably a human.

In an eleventh aspect, the invention features a use of the polypeptide described in the first aspect for treatment of metabolic-related diseases and disorders and/or reducing or increasing body mass. Preferably, said metabolic-related diseases and disorders are selected from the group consisting of obesity, insulin resistance, atherosclerosis, atheromatous disease, heart disease, hypertension, stroke, Syndrome X, non-insulin-dependent diabetes and Type II diabetes. Type II diabetes-related complications to be treated by the methods of the invention include microangiopathic lesions, ocular lesions, and renal lesions. Heart disease includes, but is not limited to, cardiac insufficiency, coronary insufficiency, and high blood pressure. Other metabolic-related disorders to be treated by compounds of the invention include hyperlipidemia and hyperuricemia. Yet other metabolic-related diseases or disorders of the invention include cachexia, wasting, AIDS-related weight loss, anorexia, and bulimia. In preferred embodiments, said individual is a mammal, preferably a human.

The invention further features a use of the polypeptide described in the first aspect for prevention of weight gain, for weight reduction, and/or for maintenance of weight loss. In preferred embodiments, said individual is a mammal, preferably a human.

In a twelfth aspect, the invention provides a polypeptide of the first aspect of the invention, or a composition of the fifth aspect of the invention, for use in a method of treatment of the human or animal body.

In a thirteenth aspect, the invention features methods of reducing body weight for cosmetic purposes comprising providing to an individual said pharmaceutical or physiologically acceptable composition described in the fifth aspect, or a polypeptide described in the first aspect. Preferably, for said reducing body weight said individual has a BMI of at least 20 and no more than 25. Alternatively, for said increasing body weight said individual preferably has a BMI of at least 15 and no more than 20.

In a related aspect, the invention features methods of maintaining weight loss comprising providing to an individual said pharmaceutical or physiologically acceptable composition described in the fifth aspect, or the polypeptide described in the first aspect. Where the maintenance of weight loss is practiced for cosmetic purposes, the individual has a BMI of at least 20 and no more than 25. In embodiments for the treatment of obesity by means of maintaining weight loss, the individual may have a BMI of at least 20. One embodiment for the treatment of obesity by means of maintaining weight loss provides for the treatment of individuals with BMI values of at least 25. Another embodiment for the treatment of obesity by means of maintaining weight loss provides for the treatment of individuals with BMI values of at least 30.

In a fourteenth aspect, the invention features the pharmaceutical or physiologically acceptable composition described in the fifth aspect for reducing body mass and/or for treatment or prevention of metabolic-related diseases or disorders. Preferably, said metabolic-related disease or disorder is selected from the group consisting of obesity, impaired glucose tolerance, insulin resistance, atherosclerosis, atheromatous disease, heart disease, hypertension, stroke, Syndrome X, non-insulin-dependent diabetes and Type II diabetes. Type II diabetes-related complications to be treated by the methods of the invention include microangiopathic lesions, ocular lesions, and renal lesions. Heart disease includes, but is not limited to, cardiac insufficiency, coronary insufficiency, and high blood pressure. Other metabolic-related disorders to be treated by compounds of the invention include hyperlipidemia and hyperuricemia. Yet other metabolic-related diseases or disorders of the invention include cachexia, wasting, AIDS-related weight loss, cancer-related weight loss, anorexia, and bulimia. In preferred embodiments, said individual is a mammal, preferably a human. In preferred embodiments, the identification of said individuals to be treated with said pharmaceutical or physiologically acceptable composition comprises genotyping XCRF single nucleotide polymorphisms (SNPs) or measuring XCRF polypeptides or mRNA levels in clinical samples from said individuals. Preferably, said clinical samples are selected from the group consisting of blood, serum, plasma, urine, and saliva.

In a fifteenth aspect, the invention features the pharmaceutical or physiologically acceptable composition described in the fifth aspect for reducing body weight for cosmetic reasons.

In a related aspect, the invention features the pharmaceutical or physiologically acceptable composition described in the fifth aspect for maintaining weight loss for cosmetic reasons.

In a sixteenth aspect, the invention features methods of treating insulin resistance comprising providing to an individual said pharmaceutical or physiologically acceptable composition described in the fifth aspect, or a polypeptide described in the first aspect.

In a seventeenth aspect, the invention features the pharmaceutical or physiologically acceptable composition described in the fifth aspect in a method of treating individuals with normal glucose tolerance (NGT) who are obese or who have fasting hyperinsulinemia, or who have both.

In further preferred embodiments, the invention features the pharmaceutical or physiologically acceptable composition described in the fifth aspect in a method of treating individuals with gestational diabetes. Gestational diabetes refers to the development of diabetes in an individual during pregnancy, usually during the second or third trimester of pregnancy.

In further preferred embodiments, the invention features the pharmaceutical or physiologically acceptable composition described in the fifth aspect in a method of treating individuals with impaired fasting glucose (IFG). Impaired fasting glucose (IFG) is that condition in which fasting plasma glucose levels in an individual are elevated but not diagnostic of overt diabetes, i.e. plasma glucose levels of less than 126 mg/dl and less than or equal to 110 mg/dl.

In further preferred embodiments, the invention features the pharmaceutical or physiologically acceptable composition described in the fifth aspect in a method of treating and preventing impaired glucose tolerance (IGT) in an individual. By providing therapeutics and methods for reducing or preventing IGT, i.e., for normalizing insulin resistance, the progression to NIDDM can be delayed or prevented. Furthermore, by providing therapeutics and methods for reducing or preventing insulin resistance, the invention provides methods for reducing and/or preventing the appearance of Insulin-Resistance Syndrome.

In further preferred embodiments, the invention features the pharmaceutical or physiologically acceptable composition described in the fifth aspect in a method of treating a subject having polycystic ovary syndrome (PCOS). PCOS is among the most common disorders of premenopausal women, affecting 5-10% of this population. Insulin-sensitizing agents, e.g., troglitazone, have been shown to be effective in PCOS and that, in particular, the defects in insulin action, insulin secretion, ovarian steroidogenosis and fibrinolysis are improved (Ehrman et al. (1997) J Clin Invest 100:1230), such as in insulin-resistant humans. Accordingly, the invention provides methods for reducing insulin resistance, normalizing blood glucose thus treating and/or preventing PCOS.

In further preferred embodiments, the invention features the pharmaceutical or physiologically acceptable composition described in the fifth aspect in a method of treating a subject having insulin resistance.

In further preferred embodiments, a subject having insulin resistance is treated according to the

methods of the invention to reduce or cure the insulin-resistance. As insulin resistance is also often associated with infections and cancer, prevention or reducing insulin resistance according to the methods of the invention may prevent or reduce infections and cancer.

In further preferred embodiment, the methods of the invention are used to prevent the development of insulin resistance in a subject, e.g., those known to have an increased risk of developing insulin-resistance.

Thus, any of the above-described tests or other tests known in the art can be used to determine that a subject is insulin-resistant, which patient can then be treated according to the methods of the invention to reduce or cure the insulin-resistance. Alternatively, the methods of the invention can also be used to prevent the development of insulin resistance in a subject, e.g., those known to have an increased risk of developing insulin-resistance.

In an eighteenth aspect, the invention features a method of preventing or treating an metabolic-related disease or disorder comprising providing or administering to an individual in need of such treatment said pharmaceutical or physiologically acceptable composition described in the fifth aspect. In preferred embodiments, the identification of said individuals in need of such treatment to be treated with said pharmaceutical or physiologically acceptable composition comprises genotyping XCRF single nucleotide polymorphisms (SNPs) or measuring XCRF polypeptide or mRNA levels in clinical samples from said individuals. Preferably, said clinical samples are selected from the group consisting of blood, serum, plasma, urine, and saliva. Preferably, said metabolic-related disease or disorder is selected from the group consisting of obesity, impaired glucose tolerance, insulin resistance, atherosclerosis, atheromatous disease, heart disease, hypertension, stroke, Syndrome X, non-insulin-dependent diabetes and Type II diabetes. Type II diabetes-related complications to be treated by the methods of the invention include microangiopathic lesions, ocular lesions, and renal lesions. Heart disease includes, but is not limited to, cardiac insufficiency, coronary insufficiency, and high blood pressure. Other metabolic-related disorders to be treated by compounds of the invention include hyperlipidemia and hyperuricemia. Yet other metabolic-related diseases or disorders of the invention include cachexia, wasting, AIDS-related weight loss, cancer-related weight loss, anorexia, and bulimia. In preferred embodiments, said individual is a mammal, preferably a human.

In a nineteenth aspect, the invention features a method of using an XCRF polypeptide to screen compounds for one or more antagonists of XCRF polypeptide activity, wherein said activity is selected from but not restricted to lipid partitioning, lipid metabolism, and insulin-like activity.

In a related aspect, the invention features a method of using an XCRF polypeptide to screen compounds for one or more antagonists of XCRF polypeptide activity, wherein said activity is selected from but not restricted to prevention of weight gain, weight reduction, and maintenance of weight loss.

In preferred embodiment, said compound is selected from but is not restricted to small molecular weight organic or inorganic compound, protein, peptide, carbohydrate, or lipid.

In a twentieth aspect, the invention features a method of using an XCRF polypeptide to identify one or more cell types expressing a cell surface receptor for said XCRF polypeptide, preferably wherein said polypeptide comprises all or part of the globular C-terminal C1q homology domain and has lipid partitioning, lipid metabolism, or insulin-like activities.

In a twenty-first aspect, the invention features a method of using an XCRF polypeptide to clone cDNA encoding a cell surface receptor for said XCRF polypeptide, preferably wherein said polypeptide comprises all or part of the globular C-terminal C1q homology domain and has lipid partitioning, lipid metabolism, or insulin-like activities.

In a twenty-second aspect, the invention features a method of using an XCRF polynucleotide to generate transgenic non-human mammals expressing XCRF polypeptides, preferably wherein said non-human mammal is mouse, cow, sheep, goat, pig, or rabbit.

In a twenty-third aspect, the invention features a purified or isolated antibody capable of specifically binding to a polypeptide of the present invention. In one aspect of this embodiment, the antibody is capable of binding to a polypeptide comprising at least 6 consecutive amino acids, at least 8 consecutive amino acids, or at least 10 consecutive amino acids of the polypeptide of SEQ ID NO: 2, 4, 6 or 8.

In a preferred aspect of the methods above and disclosed herein, the amount of XCRFpolypeptide or polynucleotide administered to an individual is sufficient to bring circulating (blood, serum, or plasma) levels (concentration) of XCRF polypeptides to their normal levels (levels in non-obese individuals). "Normal levels" may be specified as the total concentration of all circulating XCRF polypeptides (full-length XCRF proteins and fragments thereof) or the concentration of all circulating proteolytically cleaved XCRF polypeptides only.

In a further preferred aspect of the methods above and disclosed herein, weight loss is due in part or in whole to a decrease in mass of either a) subcutaneous adipose tissue and/or b) visceral (omental) adipose tissue.

Full-length XCRF polypeptides and polynucleotides encoding the same may be specifically substituted for an XCRF polypeptide fragment or polynucleotide encoding the same in any embodiment of the present invention.

DETAILED DESCRIPTION OF THE SEQUENCE LISTING

SEQ ID NO:1 represents the cDNA sequence of XCRF1.

SEQ ID NO:2 represents the amino acid sequence encoded by the cDNA of SEQ ID NO:1.

SEQ ID NO:3 represents the cDNA sequence of XCRF3.

SEQ ID NO:4 represents the amino acid sequence encoded by the cDNA of SEQ ID NO:3.

SEQ ID NO:5 represents the cDNA sequence of XCRF4.

SEQ ID NO:2 represents the amino acid sequence encoded by the cDNA of SEQ ID NO:5.

SEQ ID NO:7 represents the cDNA sequence of XCRF5.

SEQ ID NO:8 represents the amino acid sequence encoded by the cDNA of SEQ ID NO:7.

DETAILED DESCRIPTION OF THE INVENTION

Before describing the invention in greater detail, the following definitions are set forth to illustrate and define the meaning and scope of the terms used to describe the invention herein.

The term "XCRF" or "XCRF", as used herein, encompasses any one or more of the molecules designated XCRF1, XCRF3, XCRF4 and XCRF5, as well as fragments, salts, active fractions, isoforms, muteins or functional derivatives thereof.

As used interchangeably herein, the terms "oligonucleotides", and "polynucleotides" and nucleic acid include RNA, DNA, or RNA/DNA hybrid sequences of more than one nucleotide in either single chain or duplex form. The terms encompass "modified nucleotides" which comprise at least one modification, including by way of example and not limitation: (a) an alternative linking group, (b) an analogous form of purine, (c) an analogous form of pyrimidine, or (d) an analogous sugar. For examples of analogous linking groups, purines, pyrimidines, and sugars see for example PCT publication No. WO 95/04064. The polynucleotide sequences of the invention may be prepared by any known method, including synthetic, recombinant, *ex vivo* generation, or a combination thereof, as well as utilizing any purification methods known in the art.

The terms polynucleotide construct, recombinant polynucleotide and recombinant polypeptide are used herein consistently with their use in the art. The terms "upstream" and "downstream" are also used herein consistently with their use in the art. The terms "base paired" and "Watson & Crick base paired" are used interchangeably herein and consistently with their use in the art. Similarly, the terms "complementary", "complement thereof", "complement", "complementary polynucleotide", "complementary nucleic acid" and "complementary nucleotide sequence" are used interchangeably herein and consistently with their use in the art.

The term "purified" is used herein to describe a polynucleotide or polynucleotide vector of the invention that has been separated from other compounds including, but not limited to, other nucleic acids, carbohydrates, lipids and proteins (such as the enzymes used in the synthesis of the polynucleotide). Purified can also refer to the separation of covalently closed polynucleotides from linear polynucleotides, or vice versa, for example. A polynucleotide is substantially pure when at least

about 50%, 60%, 75%, or 90% of a sample contains a single polynucleotide sequence. In some cases this involves a determination between conformations (linear versus covalently closed). A substantially pure polynucleotide typically comprises about 50, 60, 70, 80, 90, 95, 99% weight/weight of a nucleic acid sample. Polynucleotide purity or homogeneity may be indicated by a number of means well known in the art, such as agarose or polyacrylamide gel electrophoresis of a sample, followed by visualizing a single polynucleotide band upon staining the gel. For certain purposes, higher resolution can be achieved by using HPLC or other means well known in the art.

Similarly, the term "purified" is used herein to describe a polypeptide of the invention that has been separated from other compounds including, but not limited to, nucleic acids, lipids, carbohydrates and other proteins. In some preferred embodiments, a polypeptide is substantially pure when at least about 50%, 60%, 75%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 99.5% of the polypeptide molecules of a sample have a single amino acid sequence. In some preferred embodiments, a substantially pure polypeptide typically comprises about 50%, 60%, 70%, 80%, 90% 95%, 96%, 97%, 98%, 99% or 99.5% weight/weight of a protein sample. Polypeptide purity or homogeneity is indicated by a number of methods well known in the art, such as agarose or polyacrylamide gel electrophoresis of a sample, followed by visualizing a single polypeptide band upon staining the gel. For certain purposes, higher resolution can be achieved by using HPLC or other methods well known in the art.

Further, as used herein, the term "purified" does not require absolute purity; rather, it is intended as a relative definition. Purification of starting material or natural material to at least one order of magnitude, preferably two or three orders, and more preferably four or five orders of magnitude is expressly contemplated. Alternatively, purification may be expressed as "at least" a percent purity relative to heterologous polynucleotides (DNA, RNA or both) or polypeptides. As a preferred embodiment, the polynucleotides or polypeptides of the present invention are at least; 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 96%, 96%, 98%, 99%, 99.5% or 100% pure relative to heterologous polynucleotides or polypeptides. As a further preferred embodiment the polynucleotides or polypeptides have an "at least" purity ranging from any number, to the thousandth position, between 90% and 100% (e.g., at least 99.995% pure) relative to heterologous polynucleotides or polypeptides. Additionally, purity of the polynucleotides or polypeptides may be expressed as a percentage (as described above) relative to all materials and compounds other than the carrier solution. Each number, to the thousandth position, may be claimed as individual species of purity.

The term "isolated" requires that the material be removed from its original environment (*e.g.*, the natural environment if it is naturally occurring). For example, a naturally occurring polynucleotide or polypeptide present in a living animal is not isolated, but the same polynucleotide or DNA or polypeptide, separated from some or all of the coexisting materials in the natural system, is isolated.

Such polynucleotide could be part of a vector and/or such polynucleotide or polypeptide could be part of a composition, and still be isolated in that the vector or composition is not part of its natural environment.

Specifically excluded from the definition of "isolated" are: naturally occurring chromosomes (*e.g.*, chromosome spreads), artificial chromosome libraries, genomic libraries, and cDNA libraries that exist either as an *in vitro* nucleic acid preparation or as a transfected/transformed host cell preparation, wherein the host cells are either an *in vitro* heterogeneous preparation or plated as a heterogeneous population of single colonies. Also specifically excluded are the above libraries wherein a 5' EST makes up less than 5% (or alternatively 1%, 2%, 3%, 4%, 10%, 25%, 50%, 75%, or 90%, 95%, or 99%) of the number of nucleic acid inserts in the vector molecules. Further specifically excluded are whole cell genomic DNA or whole cell RNA preparations (including said whole cell preparations which are mechanically sheared or enzymatically digested). Further specifically excluded are the above whole cell preparations as either an *in vitro* preparation or as a heterogeneous mixture separated by electrophoresis (including blot transfers of the same) wherein the polynucleotide of the invention have not been further separated from the heterologous polynucleotides in the electrophoresis medium (*e.g.*, further separating by excising a single band from a heterogeneous band population in an agarose gel or nylon blot).

The term "primer" denotes a specific oligonucleotide sequence which is complementary to a target nucleotide sequence and used to hybridize to the target nucleotide sequence. A primer serves as an initiation point for nucleotide polymerization catalyzed by DNA polymerase, RNA polymerase, or reverse transcriptase.

The term "probe" denotes a defined nucleic acid segment that can be used to identify a specific polynucleotide sequence present in a sample, said nucleic acid segment comprising a nucleotide sequence complementary to the specific polynucleotide sequence to be identified.

The term "polypeptide" refers to a polymer of amino acids without regard to the length of the polymer. Thus, peptides, oligopeptides, and proteins are included within the definition of polypeptide. This term also does not specify or exclude post-expression modifications of polypeptides. For example, polypeptides that include the covalent attachment of glycosyl groups, acetyl groups, phosphate groups, lipid groups and the like are expressly encompassed by the term polypeptide. Also included within the definition are polypeptides which contain one or more analogs of an amino acid (including, for example, non-naturally occurring amino acids, amino acids which only occur naturally in an unrelated biological system, modified amino acids from mammalian systems etc.), polypeptides with substituted linkages, as well as other modifications known in the art, both naturally occurring and non-naturally occurring.

Without being limited by theory, the compounds/polypeptides of the invention are capable of modulating the partitioning of dietary lipids between the liver and peripheral tissues, and thus of treating

"diseases involving the partitioning of dietary lipids between the liver and peripheral tissues. " The term "peripheral tissues" is meant to include muscle and adipose tissue. In preferred embodiments, the compounds/polypeptides of the invention partition the dietary lipids toward the muscle. In alternative preferred embodiments, the dietary lipids are partitioned toward the adipose tissue. In other preferred embodiments, the dietary lipids are partitioned toward the liver. In yet other preferred embodiments, the compounds/polypeptides of the invention increase or decrease the oxidation of dietary lipids, preferably free fatty acids (FFA) by the muscle. Dietary lipids include, but are not limited to triglycerides and free fatty acids.

Preferred diseases believed to involve the partitioning of dietary lipids include obesity and obesity-related diseases and disorders such as obesity, impaired glucose tolerance, insulin resistance, atherosclerosis, atheromatous disease, heart disease, hypertension, stroke, Syndrome X, Non-Insulin Dependent Diabetes Mellitus (NIDDM, or Type II diabetes) and Insulin Dependent Diabetes Mellitus (IDDM or Type I diabetes). Diabetes-related complications to be treated by the methods of the invention include microangiopathic lesions, ocular lesions, retinopathy, neuropathy, and renal lesions. Heart disease includes, but is not limited to, cardiac insufficiency, coronary insufficiency, and high blood pressure. Other obesity-related disorders to be treated by compounds of the invention include hyperlipidemia and hyperuricemia. Yet other obesity-related diseases or disorders of the invention include cachexia, wasting, AIDS-related weight loss, cancer-related weight loss, anorexia, and bulimia.

The term "heterologous", when used herein, is intended to designate any polypeptide or polynucleotide other than an XCRF polypeptide or a polynucleotide encoding an XCRF polypeptide of the present invention.

The terms "comprising", "consisting of" and "consisting essentially of" are defined according to their standard meaning. A defined meaning set forth in the M.P.E.P. controls over a defined meaning in the art and a defined meaning set forth in controlling Federal Circuit case law controls over a meaning set forth in the M.P.E.P. With this in mind, the terms may be substituted for one another throughout the instant application in order to attach the specific meaning associated with each term.

The term "host cell recombinant for" a particular polynucleotide of the present invention, means a host cell that has been altered by the hands of man to contain said polynucleotide in a way not naturally found in said cell. For example, said host cell may be transiently or stably transfected or transduced with said polynucleotide of the present invention.

The term "obesity" as used herein is defined in the WHO classifications of weight (Kopelman (2000) Nature 404:635643). Underweight is less than 18.5 (thin); Healthy is 18.5-24.9 (normal); grade 1 overweight is 25.0-29.9 (overweight); grade 2 overweight is 30.0-39.0 (obesity); grade 3 overweight is greater than or equal to 40.0 BMI. BMI is body mass index (morbid obesity) and is kg/m^2. Waist

circumference can also be used to indicate a risk of metabolic complications where in men a circumference of greater than or equal to 94 cm indicates an increased risk, and greater than or equal to 102 cm indicates a substantially increased risk. Similarly for women, greater than or equal to 88 cm indicates an increased risk, and greater than or equal to 88 cm indicates a substantially increased risk. The waist circumference is measured in cm at midpoint between lower border of ribs and upper border of the pelvis. Other measures of obesity include, but are not limited to, skinfold thickness which is a measurement in cm of skinfold thickness using calipers, and bioimpedance, which is based on the principle that lean mass conducts current better than fat mass because it is primarily an electrolyte solution; measurement of resistance to a weak current (impedance) applied across extremities provides an estimate of body fat using an empirically derived equation.

The term "energy intake" as used herein is defined as the energy introduced into an individual from total caloric intake, i.e., the total energy from food and liquid diet. The term "energy expenditure" as used herein is defined as total energy expenditure (TEE), which includes resting energy expenditure (REE), the thermic effect of feeding (TEF), and activities such as exercise. Both "energy intake" and "energy expenditure" are further defined by Rosenbaum et al. [Am J Clin Nutr (2000) Jun; 71(6):1421-32), which is hereby incorporated by reference in its entirety].

The term "maintenance of weight loss" as used herein is defined as sustaining a stable weight in an individual that is 10-20% below the initial, obese weight of the individual. Preferably, the new maintained weight after weight loss is a healthy weight (as defined herein). When the maintenance of weight loss is practiced for cosmetic purposes, the individual has a BMI of at least 20 and no more than 25. As defined for the treatment of obesity by means of maintaining weight loss, the individual may have a BMI of at least 20.

The term "diabetes" as used herein is intended to encompass the usual diagnosis of diabetes made from any of the methods included, but not limited to, the following list: symptoms of diabetes (eg. polyuria, polydipsia, polyphagia) plus casual plasma glucose levels of greater than or equal to 200 mg/dl, wherein casual plasma glucose is defined any time of the day regardless of the timing of meal or drink consumption; 8 hour fasting plasma glucose levels of less than or equal to 126 mg/dl; and plasma glucose levels of greater than or equal to 200 mg/dl 2 hours following oral administration of 75 g anhydrous glucose dissolved in water.

The term "impaired glucose tolerance (IGT)" as used herein is intended to indicate that condition associated with insulin-resistance that is intermediate between frank, NIDDM and normal glucose tolerance (NGT). A high percentage of the IGT population is known to progress to NIDDM relative to persons with normal glucose tolerance (Sad et al., New Engl J Med 1988; 319:1500-6). Thus, by providing therapeutics and methods for reducing or preventing IGT, i.e., for normalizing insulin

resistance, the progression to NIDDM can be delayed or prevented. IGT is diagnosed by a procedure wherein an affected person's postprandial glucose response is determined to be abnormal as assessed by 2-hour postprandial plasma glucose levels. In this test, a measured amount of glucose is given to the patient and blood glucose levels measured regular intervals, usually every half hour for the first two hours and every hour thereafter. In a "normal" or non-IGT individual, glucose levels rise during the first two hours to a level less than 140 mg/dl and then drop rapidly. In an IGT individual, the blood glucose levels are higher and the drop-off level is at a slower rate.

The term "Insulin-Resistance Syndrome" as used herein is intended to encompass the cluster of abnormalities resulting from an attempt to compensate for insulin resistance that sets in motion a series of events that play an important role in the development of both hypertension and coronary artery disease (CAD), such as premature atherosclerotic vascular disease. Increased plasma triglyceride and decreased HDL-cholesterol concentrations, conditions that are known to be associated with CAD, have also been reported to be associated with insulin resistance. Thus, by providing therapeutics and methods for reducing or preventing insulin resistance, the invention provides methods for reducing and/or preventing the appearance of insulin-resistance syndrome.

The term "polycystic ovary syndrome (PCOS)" as used herein is intended to designate that etiologically unassigned disorder of premenopausal women, affecting 5-10% of this population, characterized by hyperandrogenism, chronic anovulation, defects in insulin action, insulin secretion, ovarian steroidogenesis and fibrinolysis. Women with PCOS frequently are insulin resistant and at increased risk to develop glucose intolerance or NIDDM in the third and fourth decades of life (Dunaif et al. (1996) J Clin Endocrinol Metab 81:3299). Hyperandrogenism also is a feature of a variety of diverse insulin-resistant states, from the type A syndrome, through leprechaunism and lipoatrophic diabetes, to the type B syndrome, when these conditions occur in premenopausal women. It has been suggested that hyperinsulinemia per se causes hyperandrogenism. Insulin-sensitizing agents, e.g., troglitazone, have been shown to be effective in PCOS and that, in particular, the defects in insulin action, insulin secretion, ovarian steroidogenosis and fibrinolysis are improved (Ehrman et al. (1997) J Clin Invest 100:1230), such as in insulin-resistant humans.

The term "insulin resistance" as used herein is intended to encompass the usual diagnosis of insulin resistance made by any of a number of methods, including but not restricted to: the intravenous glucose tolerance test or measurement of the fasting insulin level. It is well known that there is an excellent correlation between the height of the fasting insulin level and the degree of insulin resistance. Therefore, one could use elevated fasting insulin levels as a surrogate marker for insulin resistance for the purpose of identifying which normal glucose tolerance (NGT) individuals have insulin resistance. Another way to do this is to follow the approach as disclosed in The New England Journal of Medicine,

No. 3, pp. 1188 (1995), i.e. to select obesity as an initial criterion for entry into the treatment group. Some obese subjects have impaired glucose tolerance (IGT) while others have normal glucose tolerance (NGT). Since essentially all obese subjects are insulin resistant, i.e. even the NGT obese subjects are insulin resistant and have fasting hyperinsulinemia. Therefore, the target of the treatment according to the present invention can be defined as NGT individuals who are obese or who have fasting hyperinsulinemia, or who have both.

A diagnosis of insulin resistance can also be made using the euglycemic glucose clamp test. This test involves the simultaneous administration of a constant insulin infusion and a variable rate glucose infusion. During the test, which lasts 3-4 hours, the plasma glucose concentration is kept constant at euglycemic levels by measuring the glucose level every 5-10 minutes and then adjusting the variable rate glucose infusion to keep the plasma glucose level unchanged. Under these circumstances, the rate of glucose entry into the bloodstream is equal to the overall rate of glucose disposal in the body. The difference between the rate of glucose disposal in the basal state (no insulin infusion) and the insulin infused state, represents insulin mediated glucose uptake. In normal individuals, insulin causes brisk and large increase in overall body glucose disposal, whereas in NIDDM subjects, this effect of insulin is greatly blunted, and is only 20-30% of normal. In insulin resistant subjects with either IGT or NGT, the rate of insulin stimulated glucose disposal is about half way between normal and NIDDM. For example, at a steady state plasma insulin concentration of about 100 uU/ml (a physiologic level) the glucose disposal rate in normal subjects is about 7 mg/kg/min. In NIDDM subjects, it is about 2.5mg/.kg/min., and in patients with IGT (or insulin resistant subjects with NGT) it is about 4-5 mg/kg/min. This is a highly reproducible and precise test, and can distinguish patients within these categories. It is also known that as subjects become more insulin resistant, the fasting insulin level rises. There is an excellent positive correlation between the height of the fasting insulin level and the magnitude of the insulin resistance as measured by euglycemic glucose clamp tests and, therefore, this provides the rationale for using fasting insulin levels as a surrogate measure of insulin resistance.

The term "agent acting on the partitioning of dietary lipids between the liver and peripheral tissues" refers to a compound or polypeptide of the invention that modulates the partitioning of dietary lipids between the liver and the peripheral tissues as previously described. Preferably, the agent increases or decreases the oxidation of dietary lipids, preferably free fatty acids (FFA) by the muscle. Preferably the agent decreases or increases the body weight of individuals or is used to treat or prevent an obesity-related disease or disorder such as obesity, impaired glucose tolerance, insulin resistance, atherosclerosis, atheromatous disease, heart disease, hypertension, stroke, Syndrome X, Non-Insulin Dependent Diabetes Mellitus (NIDDM, or Type II diabetes) and Insulin Dependent Diabetes Mellitus (IDDM or Type I diabetes). Diabetes-related complications to be treated by the methods of the

invention include microangiopathic lesions, ocular lesions, retinopathy, neuropathy, renal lesions. Heart disease includes, but is not limited to, cardiac insufficiency, coronary insufficiency, and high blood pressure. Other obesity-related disorders to be treated by compounds of the invention include hyperlipidemia and hyperuricemia. Yet other obesity-related diseases or disorders of the invention include cachexia, wasting, AIDS-related weight loss, cancer-related weight loss, anorexia, and bulimia.

The terms "response to an agent acting on the partitioning of dietary lipids between the liver and peripheral tissues " refer to drug efficacy, including but not limited to, ability to metabolize a compound, ability to convert a pro-drug to an active drug, and the pharmacokinetics (absorption, distribution, elimination) and the pharmacodynamics (receptor-related) of a drug in an individual.

The terms "side effects to an agent acting on the partitioning of dietary lipids between the liver and peripheral tissues " refer to adverse effects of therapy resulting from extensions of the principal pharmacological action of the drug or to idiosyncratic adverse reactions resulting from an interaction of the drug with unique host factors. "Side effects to an agent acting on the partitioning of dietary lipids between the liver and peripheral tissues " can include, but are not limited to, adverse reactions such as dermatologic, hematologic or hepatologic toxicities and further includes gastric and intestinal ulceration, disturbance in platelet function, renal injury, nephritis, vasomotor rhinitis with profuse watery secretions, angioneurotic edema, generalized urticaria, and bronchial asthma to laryngeal edema and bronchoconstriction, hypotension, and shock.

The term "XCRF-related diseases and disorders" as used herein refers to any disease or disorder comprising an aberrant functioning of XCRF, or which could be treated or prevented by modulating XCRF levels or activity. "Aberrant functioning of XCRF" includes, but is not limited to, aberrant levels of expression of XCRF (either increased or decreased, but preferably decreased), aberrant activity of XCRF (either increased or decreased), and aberrant interactions with ligands or binding partners (either increased or decreased). By "aberrant" is meant a change from the type, or level of activity seen in normal cells, tissues, or patients, or seen previously in the cell, tissue, or patient prior to the onset of the illness. In preferred embodiments, these XCRF-related diseases and disorders include obesity and the metabolic-related diseases and disorders described previously.

The term "cosmetic treatments" is meant to include treatments with compounds or polypeptides of the invention that increase or decrease the body mass of an individual where the individual is not clinically obese or clinically thin. Thus, these individuals have a body mass index (BMI) below the cut-off for clinical obesity (*e.g.* below 25 kg/m^2) and above the cut-off for clinical thinness (*e.g.* above 18.5 kg/m^2). In addition, these individuals are preferably healthy (*e.g.* do not have an metabolic-related disease or disorder of the invention). "Cosmetic treatments" are also meant to encompass, in some circumstances, more localized increases in adipose tissue, for example, gains or losses specifically

around the waist or hips, or around the hips and thighs, for example. These localized gains or losses of adipose tissue can be identified by increases or decreases in waist or hip size, for example.

The term "preventing" as used herein refers to administering a compound prior to the onset of clinical symptoms of a disease or condition so as to prevent a physical manifestation of aberrations associated with obesity or XCRF.

The term "treating" as used herein refers to administering a compound after the onset of clinical symptoms.

The term "in need of treatment" as used herein refers to a judgment made by a caregiver (*e.g.* physician, nurse, nurse practitioner, etc in the case of humans; veterinarian in the case of animals, including non-human mammals) that an individual or animal requires or will benefit from treatment. This judgment is made based on a variety of factors that are in the realm of a caregiver's expertise, but that include the knowledge that the individual or animal is ill, or will be ill, as the result of a condition that is treatable by the compounds of the invention.

The term "perceives a need for treatment" refers to a sub-clinical determination that an individual desires to reduce weight for cosmetic reasons as discussed under "cosmetic treatment" above. The term "perceives a need for treatment" in other embodiments can refer to the decision that an owner of an animal makes for cosmetic treatment of the animal.

The term "individual" or "patient" as used herein refers to any animal, including mammals, preferably mice, rats, other rodents, rabbits, dogs, cats, swine, cattle, sheep, horses, or primates, and most preferably humans. The term may specify male or female or both, or exclude male or female.

The term "non-human animal" refers to any non-human vertebrate, including birds and more usually mammals, preferably primates, animals such as swine, goats, sheep, donkeys, horses, cats, dogs, rabbits or rodents, more preferably rats or mice. Both the terms "animal" and "mammal" expressly embrace human subjects unless preceded with the term "non-human".

XCRF polypeptides are able to significantly reduce the postprandial response of plasma free fatty acids, glucose, and triglycerides in mammals fed a high fat/sucrose meal, while not affecting levels of leptin, insulin or glucagon. In addition, XCRF polypeptides modulate muscle free fatty acid oxidation *in vitro* and *ex vivo*, preferably increase oxidation. Further, XCRF polypeptides of the invention modulate weight gain in mammals that are fed a high fat/sucrose diet.

The instant invention encompasses the use of XCRF polypeptides in the partitioning of free fatty acid (FFA) and as an important new tool to control energy homeostasis. Of the tissues that can significantly remove lipids from circulation and cause FFA oxidation, muscle is believed to be quantitatively the most important.

PREFERRED EMBODIMENTS OF THE INVENTION

The instant invention is based on XCRF1, XCRF3, XCRF4 and XCRD5, which may in the following be commonly designated "XCRF" or "XCRFs". XCRFs are of human origin. XCRF full-length polypeptide is comprised of a globular C-terminal C1q homology domain preceded by a collagen-like region, an N-terminal unique region, and a putative signal peptide. XCRF polypeptide is able to lower circulating (either blood, serum, or plasma) levels (concentration) of: (i) free fatty acids, (ii) glucose, and/or (iii) triglycerides. XCRF polypeptide is further able to (i) prevent weight gain, (ii) reduce weight, and/or (iii) maintain weight loss.

In particular, the invention relates to the use of XCRF for the preparation of a medicament for treating and/or preventing a metabolic disease, wherein XCRF is selected from

a) A polypeptide comprising SEQ ID NO: 2;
b) A polypeptide comprising amino acids 17 to 258 of SEQ ID NO: 2;
c) A polypeptide comprising SEQ ID NO: 4;
d) A polypeptide comprising amino acids 22 to 287 of SEQ ID NO: 4;
e) A polypeptide comprising SEQ ID NO: 6;
f) A polypeptide comprising amino acids 16 to 238 of SEQ ID NO: 6;
g) A polypeptide comprising SEQ ID NO: 8;
h) A polypeptide comprising amino acids 22 to 287 of SEQ ID NO: 8;
i) A fragment of any of (a) to (h) comprising the C-terminal C1q homology domain;
j) A mutein of any of (a) to (i), wherein the amino acid sequence has at least 40 % or 50 % or 60 % or 70 % or 80 % or 90 % identity to at least one of the sequences in (a) to (i);
k) A mutein of any of (a) to (i) which is encoded by a DNA sequence which hybridizes to the complement of the native DNA sequence encoding any of (a) to (i) under moderately stringent conditions or under high stringent conditions;
l) A mutein of any of (a) to (i) wherein any changes in the amino acid sequence are conservative amino acid substitutions to the amino acid sequences in (a) to (i);
m) a salt or an isoform, fused protein, functional derivative, active fraction or circularly permutated derivative of any of (a) to (i).

In a preferred embodiment, said metabolic disease is selected from the group consisting of:

a) obesity;
b) impaired glucose tolerance;
c) insulin resistance;
d) Syndrome X;
e) atherosclerosis; and

f) Type II diabetes.

In a further preferred embodiment, the XCRF is glycosylated at one or more sites.

In an alternative embodiment, the substance is not glycosylated.

The fused protein may comprise an immunoglobulin (Ig) fusion.

The functional derivative may comprise at least one moiety attached to one or more functional groups which occur as one or more side chains on the amino acid residues.

In a preferred embodiment, the moiety is a polyethylene moiety.

In a further aspect, the invention relates to the use of a nucleic acid molecule for manufacture of a medicament for the treatment and/or prevention of a metabolic disease, wherein the nucleic acid molecule comprises a nucleic acid sequence encoding a polypeptide selected from:

a) A polypeptide comprising SEQ ID NO: 2;
b) A polypeptide comprising amino acids 17 to 258 of SEQ ID NO: 2;
c) A polypeptide comprising SEQ ID NO: 4;
d) A polypeptide comprising amino acids 22 to 287 of SEQ ID NO: 4;
e) A polypeptide comprising SEQ ID NO: 6;
f) A polypeptide comprising amino acids 16 to 238 of SEQ ID NO: 6;
g) A polypeptide comprising SEQ ID NO: 8;
h) A polypeptide comprising amino acids 22 to 287 of SEQ ID NO: 8;
i) A fragment of any of (a) to (h) comprising the C-terminal C1q homology domain;
j) A mutein of any of (a) to (i), wherein the amino acid sequence has at least 40 % or 50 % or 60 % or 70 % or 80 % or 90 % identity to at least one of the sequences in (a) to (i);
k) A mutein of any of (a) to (i) which is encoded by a DNA sequence which hybridizes to the complement of the native DNA sequence encoding any of (a) to (i) under moderately stringent conditions or under high stringent conditions;
l) A mutein of any of (a) to (i) wherein any changes in the amino acid sequence are conservative amino acid substitutions to the amino acid sequences in (a) to (i);
m) an isoform, fused protein, active fraction or circularly permutated derivative of any of (a) to (i).

In a further aspect, the invention relates to the use of a vector comprising said nucleic acid molecule for the manufacture of a medicament for treatment and/or prevention of a metabolic disease.

The vector may preferably be an expression vector. It may further be a gene therapy vector.

Use of a vector for inducing and/or enhancing the endogenous production of a polypeptide according to any of claims 2 to 5 in a cell for the preparation of a medicament for the treatment and/or prevention of a metabolic disorder.

In yet a further aspect, the invention relates to the use of a cell comprising a vector according to the invention for the preparation of a medicament for the treatment and/or prevention of a metabolic disorder. Preferably, the metabolic diseases is selected from:

a) obesity;
b) impaired glucose tolerance;
c) insulin resistance;
d) Syndrome X;
e) atherosclerosis; and
f) Type II diabetes.

I. XCRF Polypeptides of the Invention

XCRF polypeptides have been identified that have measurable activity *in vitro* and *in vivo*. These activities include, but are not limited to, modulation, preferably reduction, of the postprandial response of plasma free fatty acids, glucose, and triglycerides in mammals fed a high fat/sucrose meal (Example 6), change, preferably an increase, in muscle free fatty acid oxidation *in vitro* and *ex vivo* (Example 10), and sustained weight loss in mammals on a high fat/sucrose diet. Other assays for XCRF polypeptide activity *in vitro* and *in vivo* are also provided (Examples 2, 5, 7, 9, 11, for example), and equivalent assays can be designed by those with ordinary skill in the art.

The term "XCRF polypeptides" includes both the "full-length" polypeptide and fragments of the "full-length" XCRF polypeptides (although each of the above species may be particularly specified).

By "intact" or "full-length" XCRF polypeptides as used herein is meant the full-length polypeptide sequence of any XCRF polypeptide, from the N-terminal methionine to the C-terminal stop codon. Examples of intact or full-length XCRF polypeptides are found in the sequence listing.

The term "metabolic-related activity" as used herein refers to at least one, and preferably all, of the activities described herein for XCRF polypeptides. Assays for the determination of these activities are provided herein (*e.g.* Examples 2-14), and equivalent assays can be designed by those with ordinary skill in the art. Optionally, "metabolic-related activity" can be selected from the group consisting of lipid partitioning, lipid metabolism, and insulin-like activity, or an activity within one of these categories. By "lipid partitioning" activity is meant the ability to effect the location of dietary lipids among the major tissue groups including, adipose tissue, liver, and muscle. XCRF polypeptides of the invention play a role in the partitioning of lipids to the muscle, liver or adipose tissue. By "lipid metabolism" activity is meant the ability to influence the metabolism of lipids. XCRF polypeptides of the invention have the ability to affect the level of free fatty acids in the plasma as well as to modulate, preferably increase, the metabolism of lipids in the muscle through free fatty acid oxidation experiments (Examples 2, 6, 8, 9, 10) and to transiently affect the levels of triglycerides in the plasma and the muscle

(Examples 6, 8, 11). By "insulin-like" activity is meant the ability of XCRF polypeptides to modulate the levels of glucose in the plasma. XCRF polypeptides do not significantly impact insulin levels but do impact glucose levels similarly to the effects of insulin (Examples 7 & 8). These effects may vary in the presence of the intact (full-length) XCRF polypeptides or are significantly greater in the presence of the XCRF polypeptide fragments compared with the full-length XCRF polypeptides.

The term "significantly greater " as used herein refers to a comparison of the activity of an XCRF polypeptide in an metabolic-related assay compared with untreated cells in the same assay. By "significantly" as used herein is meant statistically significant as it is typically determined by those with ordinary skill in the art. For example, data are typically calculated as a mean ± SEM, and a p-value $\leq$ 0.05 is considered statistically significant. Statistical analysis is typically done using either the unpaired Student's t test or the paired Student's t test, as appropriate in each study. Examples of a significant change in activity as a result of the presence of an XCRF polypeptide of the invention compared to untreated cells include an increase or a decrease in a given parameter of at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, or 75%. One or more, but not necessarily all, of the measurable parameters will change significantly in the presence of XCRF polypeptide as compared to untreated cells.

Representative "metabolic-related assays" are provided in the Examples. These assays include, but are not limited to, methods of measuring the postprandial response, methods of measuring free fatty acid oxidation, and methods of measuring weight modulation. In preferred embodiments, the post-prandial response is measured in non-human animals, preferably mice. In preferred embodiments changes in dietary lipids are measured, preferably free fatty acids and/or triglycerides. In other embodiments, other physiologic parameters are measured including, but not limited to, levels of glucose, insulin, and leptin. In other preferred embodiments, free fatty acid oxidation is measured in cells *in vitro* or *ex vivo,* preferably in muscle cells or tissue of non-human animals, preferably mice. In yet other preferred embodiments weight modulation is measured in human or non-human animals, preferably rodents (rats or mice), primates, canines, felines or procines. on a high fat/sucrose diet. Optionally, "metabolic-related activity" includes other activities not specifically identified herein. In general, "measurable parameters" relating to obesity and the field of metabolic research can be selected from the group consisting of free fatty acid levels, free fatty acid oxidation, triglyceride levels, glucose levels, insulin levels, leptin levels, food intake, weight, leptin and lipoprotein binding, uptake and degradation and lipolysis stimulated receptor (LSR) expression.

In these metabolic-related assays, preferred XCRF polypeptides would cause a significant change in at least one of the measurable parameters selected from the group consisting of post-prandial lipemia, free fatty acid levels, triglyceride levels, glucose levels, free fatty acid oxidation, and weight.

Alternatively, preferred XCRF polypeptides would have a significant change in at least one of the measurable parameters selected from the group consisting of an increase in LSR activity, an increase in leptin activity and an increase in lipoprotein activity. By "LSR" activity is meant expression of LSR on the surface of the cell, or in a particular conformation, as well as its ability to bind, uptake, and degrade leptin and lipoprotein. By "leptin" activity is meant its binding, uptake and degradation by LSR, as well as its transport across a blood brain barrier, and potentially these occurrences where LSR is not necessarily the mediating factor or the only mediating factor. Similarly, by "lipoprotein" activity is meant its binding, uptake and degradation by LSR, as well as these occurrences where LSR is not necessarily the mediating factor or the only mediating factor.

The invention is drawn, *inter alia*, to isolated, purified or recombinant XCRF polypeptides. XCRF polypeptides of the invention are useful for reducing or, using antagonists of XCRF polypeptides, increasing body weight either as a cosmetic treatment or for treatment or prevention of metabolic-related diseases and disorders. XCRF polypeptides are also useful *inter alia* in screening assays for agonists or antagonists of XCRF polypeptide activity; for raising XCRF polypeptide-specific antibodies; and in diagnostic assays. When used for cosmetic treatments, or for the treatment or prevention of metabolic-related diseases, disorders or conditions, one or more XCRF polypeptide fragments can be provided to a subject. Thus, various fragments of the full-length protein can be combined into a "cocktail" for use in the various treatment regimens.

The full-length XCRF polypeptide is comprised of about four distinct regions including:

1. an N-terminal putative signal peptide sequence about from amino acids 1-16 of SEQ ID NO: 2 for XCRF1; or from amino acids 1 to 21 of SEQ ID NO: 4 for XCRF3; or from amino acids 1 to 15 of SEQ ID NO: 6 for XCRF4; or from amino acid 1 to 21 of SEQ ID NO: 8 for XCRF5.

2. a unique region about from amino acids 17-66 of SEQ ID NO: 2 for XCRF1; or about from amino acids 22-75 of SEQ ID NO: 4 for XCRF3; or about from amino acids 16-54 of SEQ ID NO: 6 for XCRF 4; or about from amino acids 22-75 of SEQ ID NO: 8 for XCRF5;

3. a collagen-like region about from amino acids 67-117 of SEQ ID NO: 2 for XCRF1; or about from amino acids 76-120 of SEQ ID NO: 4 for XCRF3; or about from amino acids 55-96 of SEQ ID NO: 6 for XCRF4; or about from amino acids 76-120 of SEQ ID NO: 8 for XCRF5; and

4. a globular C-terminal C1q homology domain about from amino acids 118-258 of SEQ ID NO: 2 for XCRF1; or about from amino acids 121-287 of SEQ ID NO: 4 for XCRF3; or about from amino acids 97-238 of SEQ ID NO: 6 for XCRF4; or about from amino acids 121-287 of SEQ ID NO: 8 for XCRF5.

XCRF polypeptides of the invention include variants, fragments, analogs and derivatives of the XCRF polypeptides described above, including modified XCRF polypeptides.

The XCRF polypeptides of the present invention are preferably provided in an isolated form, and may be partially or substantially purified. A recombinantly produced version of any one of the XCRF polypeptides can be substantially purified by the one-step method described by Smith et al. ((1988) Gene 67:31-40) or by the methods described herein or known in the art. Polypeptides of the invention also can be purified from natural or recombinant sources using antibodies directed against the polypeptides of the invention by methods known in the art of protein purification.

Preparations of XCRF polypeptides of the invention involving a partial purification of or selection for the XCRF polypeptides are also specifically contemplated. These crude preparations are envisioned to be the result of the concentration of cells expressing XCRF polypeptides with perhaps a few additional purification steps, but prior to complete purification of the fragment. The cells expressing XCRF polypeptides are present in a pellet, they are lysed, or the crude polypeptide is lyophilized, for example.

XCRF polypeptide fragments can be any integer in length from at least 6 consecutive amino acids to one amino acid less than a full-length XCRF polypeptide.

Thus, for the polypeptide of SEQ ID NO: 2, an XCRF1 polypeptide fragment can be any integer of consecutive amino acids from 6 to 257, for example. The term "integer" is used herein in its mathematical sense and thus representative integers include, but are not limited to: 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256 and 257.

Each XCRF1 polypeptide fragment as described above can be further specified in terms of its N-terminal and C-terminal positions. For example, every combination of a N-terminal and C-terminal position that fragments of from 6 contiguous amino acids to one amino acid less than the full-length polypeptide of SEQ ID NO: 2 could occupy, on any given intact and contiguous full-length polypeptide sequence of SEQ ID NO: 2 are included in the present invention. Thus, a 6 consecutive amino acid fragment could occupy positions selected from the group consisting of 1-6, 2-7, 3-8, 4-9, 5-10, 6-11, 7-

12, 8-13, 9-14, 10-15, 11-16, 12-17, 13-18, 14-19, 15-20, 16-21, 17-22, 18-23, 19-24, 20-25, 21-26, 22-27, 23-28, 24-29, 25-30, 26-31, 27-32, 28-33, 29-34, 30-35, 31-36, 32-37, 33-38, 34-39, 35-40, 36-41, 37-42, 38-43, 39-44, 40-45, 41-46, 42-47, 43-48, 44-49, 45-50, 46-51, 47-52, 48-53, 49-54, 50-55, 51-56, 52-57, 53-58, 54-59, 55-60, 56-61, 57-62, 58-63, 59-64, 60-65, 61-66, 62-67, 63-68, 64-69, 65-70, 66-71, 67-72, 68-73, 69-74, 70-75, 71-76, 72-77, 73-78, 74-79, 75-80, 76-81, 77-82, 78-83, 79-84, 80-85, 81-86, 82-87, 83-88, 84-89, 85-90, 86-91, 87-92, 88-93, 89-94, 90-95, 91-96, 92-97, 93-98, 94-99, 95-100, 96-101, 97-102, 98-103, 99-104, 100-105, 101-106, 102-107, 103-108, 104-109, 105-110, 106-111, 107-112, 108-113, 109-114, 110-115, 111-116, 112-117, 113-118, 114-119, 115-120, 116-121, 117-122, 118-123, 119-124, 120-125, 121-126, 122-127, 123-128, 124-129, 125-130, 126-131, 127-132, 128-133, 129-134, 130-135, 131-136, 132-137, 133-138, 134-139, 135-140, 136-141, 137-142, 138-143, 139-144, 140-145, 141-146, 142-147, 143-148, 144-149, 145-150, 146-151, 147-152, 148-153, 149-154, 150-155, 151-156, 152-157, 153-158, 154-159, 155-160, 156-161, 157-162, 158-163, 159-164, 160-165, 161-166, 162-167, 163-168, 164-169, 165-170, 166-171, 167-172, 168-173, 169-174, 170-175, 171-176, 172-177, 173-178, 174-179, 175-180, 176-181, 177-182, 178-183, 179-184, 180-185, 181-186, 182-187, 183-188, 184-189, 185-190, 186-191, 187-192, 188-193, 189-194, 190-195, 191-196, 192-197, 193-198, 194-199, 195-200, 196-201, 197-202, 198-203, 199-204, 200-205, 201-206, 202-207, 203-208, 204-209, 205-210, 206-211, 207-212, 208-213, 209-214, 210-215, 211-216, 212-217, 213-218, 214-219, 215-220, 216-221, 217-222, 218-223, 219-224, 220-225, 221-226, 222-227, 223-228, 224-229, 225-230, 226-231, 227-232, 228-233, 229-234, 230-235, 231-236, 232-237, 233-238, 234-239, 235-240, 236-241, 237-242, 238-243, 240-245, 241-246, 242-247, 243-248, 244-249, 245-250, 246-251, 247-252, 248-253, 249-254, 250-255, 251-256, 252-257 and 253-258 of a 258 consecutive amino acid fragment. A 250 consecutive amino acid fragment could occupy positions selected from the group consisting of 1-250, 2-251, 3-252, 4-253, 5-254, 6-255, 7-256, 8-257 and 9-258. Similarly, the positions occupied by all the other fragments of sizes between 6 amino acids and 257 amino acids in SEQ ID NO: 2 are included in the present invention and can also be immediately envisaged based on these two examples and therefore, are not individually listed solely for the purpose of not unnecessarily lengthening the specification. Furthermore, the positions occupied by fragments of 6 to 257 consecutive amino acids in SEQ ID NO: 2 are included in the present invention and can also be immediately envisaged based on these two examples and therefore are not individually listed solely for the purpose of not unnecessarily lengthening the specification. In addition, the positions occupied by fragments of 6 consecutive amino acids to 1 amino acid less than any other full-length XCRF1 polypeptide can also be envisaged based on these two examples and therefore are not individually listed solely for the purpose of not unnecessarily lengthening the specification.

The XCRF1 polypeptides of the present invention may alternatively be described by the formula "n to c" (inclusive); where "n" equals the N-terminal most amino acid position (as defined by the sequence listing) and "c" equals the C-terminal most amino acid position (as defined by the sequence listing) of the polypeptide; and further where "n" equals an integer between 1 and the number of amino acids of the full-length polypeptide sequence of the present invention minus 5; and where "c" equals an integer between 6 and the number of amino acids of the full-length polypeptide sequence; and where "n" is an integer smaller then "c" by at least 6. Therefore, for the sequences provided in SEQ ID NO: 2, "n" is any integer selected from the list consisting of: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252 or 253 and "c" is any integer selected from the group consisting of: 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257 or 258. Every combination of "n" and "c" positions are included as specific embodiments of the invention. Moreover, the formula "n" to "c" may be modified as '"n1 – n2" to "c1 – c2"', wherein "n1 – n2" and "c1 – c2" represent positional ranges selected from any two integers above which represent amino acid positions of the sequence listing. Alternative formulas include '"n1 – n2" to "c"' and '"n" to "c1 – c2"'. In a

preferred embodiment, XCRF1 polypeptide fragments of the invention may be described by the formula where n1=17, n2=130, and c=258 of SEQ ID NO: 2.

In preferred embodiments, XCRF1 polypeptide fragments having activity are selected from amino acids 17-258, 18-258, 19-258, 20-258, 21-258, 22-258, 23-258, 24-258, 25-258, 26-258, 27-258, 28-258, 29-258, 30-258, 31-258, 32-258, 33-258, 34-258, 35-258, 36-258, 37-258, 38-258, 39-258, 40-258, 41-258, 42-258, 43-258, 44-258, 45-258, 46-258, 47-258, 48-258, 49-258, 50-258, 51-258, 52-258, 53-258, 54-258, 55-258, 56-258, 57-258, 58-258, 59-258, 60-258, 61-258, 62-258, 63-258, 64-258, 65-258, 66-258, 67-258, 68-258, 69-258, 70-258, 71-258, 72-258, 73-258, 74-258, 75-258, 76-258, 77-258, 78-258, 79-258, 80-258, 81-258, 82-258, 83-258, 84-258, 85-258, 86-258, 87-258, 88-258, 89-258, 90-258, 91-258, 92-258, 93-258, 94-258, 95-258, 96-258, 97-258, 98-258, 99-258, 100-258, 101-258, 102-258, 103-258, 104-258, 105-258, 106-258, 107-258, 108-258, 109-258, 110-258, 111-258, 112-258, 113-258, 114-258, 115-258, 116-258, 117-258, 118-258, 119-258, 120-258, 121-258, 122-258, 123-258, 124-258, 125-258, 126-258, 127-258, 128-258, 129-258 or 130-258 of SEQ ID NO: 2, where it is understood that amino acid 17 is taken to represent the N-terminal amino acid of mature XCRF1 polypeptide absent the signal peptide.

These specific embodiments, and other polypeptide and polynucleotide fragment embodiments described herein may be modified as being "at least", "equal to", "equal to or less than", "less than", "at least __ but not greater than ___" or "from ___ to ___" a specified size or specified N-terminal and/or C-terminal positions. It is noted that all ranges used to describe any embodiment of the present invention are inclusive unless specifically set forth otherwise.

The present invention also provides for the exclusion of any individual fragment specified by N-terminal and C-terminal positions or of any fragment specified by size in amino acid residues as described above. Further, any number of fragments specified by N-terminal and C-terminal positions or by size in amino acid residues as described above may make up a polypeptide fragment in any combination and may optionally include non-XCRF1 polypeptide sequences as well.

In preferred embodiments, XCRF1 polypeptide fragments comprising all or part of the globular C-terminal C1q homology domain and having activity selected from the group consisting of lipid partitioning, lipid metabolism, and insulin-like activity are selected from amino acids 17-258, 18-258, 19-258, 20-258, 21-258, 22-258, 23-258, 24-258, 25-258, 26-258, 27-258, 28-258, 29-258, 30-258, 31-258, 32-258, 33-258, 34-258, 35-258, 36-258, 37-258, 38-258, 39-258, 40-258, 41-258, 42-258, 43-258, 44-258, 45-258, 46-258, 47-258, 48-258, 49-258, 50-258, 51-258, 52-258, 53-258, 54-258, 55-258, 56-258, 57-258, 58-258, 59-258, 60-258, 61-258, 62-258, 63-258, 64-258, 65-258, 66-258, 67-258, 68-258, 69-258, 70-258, 71-258, 72-258, 73-258, 74-258, 75-258, 76-258, 77-258, 78-258, 79-258, 80-258, 81-258, 82-258, 83-258, 84-258, 85-258, 86-258, 87-258, 88-258, 89-258, 90-258, 91-258, 92-258, 93-258,

94-258, 95-258, 96-258, 97-258, 98-258, 99-258, 100-258, 101-258, 102-258, 103-258, 104-258, 105-258, 106-258, 107-258, 108-258, 109-258, 110-258, 111-258, 112-258, 113-258, 114-258, 115-258, 116-258, 117-258, 118-258, 119-258, 120-258, 121-258, 122-258, 123-258, 124-258, 125-258, 126-258, 127-258, 128-258, 129-258 or 130-258 of SEQ ID NO: 2, where it is understood that amino acid 17 is taken to represent the N-terminal amino acid of mature XCRF polypeptide absent the signal peptide.

In more preferred embodiments, XCRF1 polypeptide fragments comprised of all or part of the globular C-terminal C1q homology domain and having activity selected from the group consisting of lipid partitioning, lipid metabolism, and insulin-like activity are selected from amino acids 17-258, 30-258, 39-258, 46-258, 72-258, 75-258, 78-258, 99-258, 100-258, 105-258, 110-258, 115-258, 118-258, 119-258, 120-258, 121-258, 122-258, 123-258, 124-258, 125-258, 126-258, 127-258, 128-258, 129-258 or 130-258 of SEQ ID NO: 2, where it is understood that amino acid 17 is taken to represent the N-terminal amino acid of mature XCRF1 polypeptide absent the signal peptide.

In yet more preferred embodiments, XCRF1 polypeptide fragments comprised of all or part of the globular C-terminal C1q homology domain and having activity are selected from amino acids 17-258, 99-258, 100-258, 105-258, 110-258, 115-258, 118-258, 119-258, 120-258, 121-258, 122-258, 123-258, 124-258, 125-258, 126-258, 127-258, 128-258, 129-258 or 130-258 of SEQ ID NO: 2, where it is understood that amino acid 17 is taken to represent the N-terminal amino acid of mature XCRF1 polypeptide absent the signal peptide.

In other preferred embodiments, said XCRF1 polypeptide fragments comprised of all or part of the globular C-terminal C1q homology domain and having activity selected from the group consisting of prevention of weight gain, weight reduction, and maintenance of weight loss are selected from amino acids 17-258, 18-258, 19-258, 20-258, 21-258, 22-258, 23-258, 24-258, 25-258, 26-258, 27-258, 28-258, 29-258, 30-258, 31-258, 32-258, 33-258, 34-258, 35-258, 36-258, 37-258, 38-258, 39-258, 40-258, 41-258, 42-258, 43-258, 44-258, 45-258, 46-258, 47-258, 48-258, 49-258, 50-258, 51-258, 52-258, 53-258, 54-258, 55-258, 56-258, 57-258, 58-258, 59-258, 60-258, 61-258, 62-258, 63-258, 64-258, 65-258, 66-258, 67-258, 68-258, 69-258, 70-258, 71-258, 72-258, 73-258, 74-258, 75-258, 76-258, 77-258, 78-258, 79-258, 80-258, 81-258, 82-258, 83-258, 84-258, 85-258, 86-258, 87-258, 88-258, 89-258, 90-258, 91-258, 92-258, 93-258, 94-258, 95-258, 96-258, 97-258, 98-258, 99-258, 100-258, 101-258, 102-258, 103-258, 104-258, 105-258, 106-258, 107-258, 108-258, 109-258, 110-258, 111-258, 112-258, 113-258, 114-258, 115-258, 116-258, 117-258, 118-258, 119-258, 120-258, 121-258, 122-258, 123-258, 124-258, 125-258, 126-258, 127-258, 128-258, 129-258 or 130-258 of SEQ ID NO: 2, where it is understood that amino acid 17 is taken to represent the N-terminal amino acid of mature XCRF1 polypeptide absent the signal peptide.

In other more preferred embodiments, XCRF1 polypeptide fragments comprised of all or part of the globular C-terminal C1q homology domain and having activity selected from the group consisting of prevention of weight gain, weight reduction, and maintenance of weight loss are selected from amino acids 17-258, 30-258, 39-258, 46-258, 72-258, 75-258, 78-258, 99-258, 100-258, 105-258, 110-258, 115-258, 118-258, 119-258, 120-258, 121-258, 122-258, 123-258, 124-258, 125-258, 126-258, 127-258, 128-258, 129-258 or 130-258 of SEQ ID NO: 2, where it is understood that amino acid 17 is taken to represent the N-terminal amino acid of mature XCRF1 polypeptide absent the signal peptide.

In other yet more preferred embodiments, XCRF1 polypeptide fragments comprised of all or part of the globular C-terminal C1q homology domain and having activity are selected from amino acids 17-258, 99-258, 100-258, 105-258, 110-258, 115-258, 118-258, 119-258, 120-258, 121-258, 122-258, 123-258, 124-258, 125-258, 126-258, 127-258, 128-258, 129-258 or 130-258 of SEQ ID NO: 2, where it is understood that amino acid 17 is taken to represent the N-terminal amino acid of mature XCRF1 polypeptide absent the signal peptide.

Preferably the XCRF1 polypeptide fragment is mammalian, preferably human or mouse, but most preferably human.

XCRF1 polypeptide fragments of the invention include variants, fragments, analogs and derivatives of the XCRF1 polypeptide fragments described above, including modified XCRF1 polypeptide fragments.

Regulated proteolytic cleavage of full-length XCRF1 polypeptides of the invention *in vivo* is believed by the inventors to lead to the effective generation *in vivo* of XCRF1 polypeptide fragments of the invention comprised of all or part of the globular C-terminal C1q homology domain and having activity selected from the group consisting of lipid partitioning, lipid metabolism, or insulin-like activity. Without wishing to be bound by any particular theory, particularly preferred XCRF1 polypeptide fragments of the invention comprised of all or part of the globular C-terminal C1q homology domain and having activity selected from the group consisting of lipid partitioning, lipid metabolism, and insulin-like activity are fragments of XCRF1 polypeptide of SEQ ID NO: 2 generated by proteolytic cleavage. Without wishing to be bound by any particular theory, other particularly preferred XCRF1 polypeptide fragments of the invention comprised of all or part of the globular C-terminal C1q homology domain and having activity selected from the group consisting of prevention of weight gain, weight reduction, and maintenance of weight loss are fragments of XCRF1 polypeptide of SEQ ID NO: 2 generated by proteolytic cleavage.

Without wishing to be bound by any particular theory, particularly preferred are XCRF1 polypeptide fragments of amino acids 110-258 or 115-258 of SEQ ID NO: 2 generated by collagenase cleavage. Without wishing to be bound by any particular theory, also particularly preferred are XCRF1

polypeptide fragments of amino acids 110-258 or 115-258 of SEQ ID NO: 2 generated by matrix metalloproteinase-1 (MMP-1) cleavage.

Without wishing to be bound by any particular theory, also particularly preferred are XCRF1 polypeptide fragments of amino acids 30-258, 39-258, 46-258, 72-258, 75-258, 78-258, 100-258, 105-258 or 130-258 of SEQ ID NO:2 generated by plasmin cleavage.

Without wishing to be bound by any particular theory, also particularly preferred are XCRF polypeptide fragments of amino acids 30-258, 39-258, 46-258, 72-258, 75-258, 78-258, 100-258, 105-258 or 130-258 of SEQ ID NO:2 generated by trypsin cleavage.

XCRF1 polypeptides of the invention include variants, fragments, analogs and derivatives of the XCRF polypeptides described above, including modified XCRF 1polypeptides.

For the polypeptide of SEQ ID NO: 4, a XCRF3 polypeptide fragment can be any integer of consecutive amino acids from 6 to 286, for example. The term "integer" is used herein in its mathematical sense and thus representative integers include, but are not limited to: 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285 and 286.

Each XCRF3 polypeptide fragment as described above can be further specified in terms of its N-terminal and C-terminal positions. For example, every combination of a N-terminal and C-terminal position that fragments of from 6 contiguous amino acids to one amino acid less than the full-length polypeptide of SEQ ID NO: 4 could occupy, on any given intact and contiguous full-length polypeptide sequence of SEQ ID NO: 4 are included in the present invention. Thus, a 6 consecutive amino acid fragment could occupy positions selected from the group consisting of 1-6, 2-7, 3-8, 4-9, 5-10, 6-11, 7-12, 8-13, 9-14, 10-15, 11-16, 12-17, 13-18, 14-19, 15-20, 16-21, 17-22, 18-23, 19-24, 20-25, 21-26, 22-27, 23-28, 24-29, 25-30, 26-31, 27-32, 28-33, 29-34, 30-35, 31-36, 32-37, 33-38, 34-39, 35-40, 36-41,

37-42, 38-43, 39-44, 40-45, 41-46, 42-47, 43-48, 44-49, 45-50, 46-51, 47-52, 48-53, 49-54, 50-55, 51-56, 52-57, 53-58, 54-59, 55-60, 56-61, 57-62, 58-63, 59-64, 60-65, 61-66, 62-67, 63-68, 64-69, 65-70, 66-71, 67-72, 68-73, 69-74, 70-75, 71-76, 72-77, 73-78, 74-79, 75-80, 76-81, 77-82, 78-83, 79-84, 80-85, 81-86, 82-87, 83-88, 84-89, 85-90, 86-91, 87-92, 88-93, 89-94, 90-95, 91-96, 92-97, 93-98, 94-99, 95-100, 96-101, 97-102, 98-103, 99-104, 100-105, 101-106, 102-107, 103-108, 104-109, 105-110, 106-111, 107-112, 108-113, 109-114, 110-115, 111-116, 112-117, 113-118, 114-119, 115-120, 116-121, 117-122, 118-123, 119-124, 120-125, 121-126, 122-127, 123-128, 124-129, 125-130, 126-131, 127-132, 128-133, 129-134, 130-135, 131-136, 132-137, 133-138, 134-139, 135-140, 136-141, 137-142, 138-143, 139-144, 140-145, 141-146, 142-147, 143-148, 144-149, 145-150, 146-151, 147-152, 148-153, 149-154, 150-155, 151-156, 152-157, 153-158, 154-159, 155-160, 156-161, 157-162, 158-163, 159-164, 160-165, 161-166, 162-167, 163-168, 164-169, 165-170, 166-171, 167-172, 168-173, 169-174, 170-175, 171-176, 172-177, 173-178, 174-179, 175-180, 176-181, 177-182, 178-183, 179-184, 180-185, 181-186, 182-187, 183-188, 184-189, 185-190, 186-191, 187-192, 188-193, 189-194, 190-195, 191-196, 192-197, 193-198, 194-199, 195-200, 196-201, 197-202, 198-203, 199-204, 200-205, 201-206, 202-207, 203-208, 204-209, 205-210, 206-211, 207-212, 208-213, 209-214, 210-215, 211-216, 212-217, 213-218, 214-219, 215-220, 216-221, 217-222, 218-223, 219-224, 220-225, 221-226, 222-227, 223-228, 224-229, 225-230, 226-231, 227-232, 228-233, 229-234, 230-235, 231-236, 232-237, 233-238, 234-239, 235-240, 236-241, 237-242, 238-243, 239-244, 240-245, 241-246, 242-247, 243-248, 244-249, 245-250, 246-251, 247-252, 248-253, 249-254, 250-255, 251-256, 252-257, 253-258, 254-259, 255-260, 256-261, 257-262, 258-263, 259-264, 260-265, 261-266, 262-267, 263-268, 264-269, 265-270, 266-271, 267-272, 268-273, 269-274, 270-275, 271-276, 272-277, 273-278, 274-279, 275-280, 276-281, 277-282, 278-283, 279-284, 280-285, 281-286 and 282-287 of a 287 consecutive amino acid polypeptide. A 280 consecutive amino acid fragment could occupy positions selected from the group consisting of 1-280, 2-281, 3-282, 4-283, 5-284, 6-285, 7-286, and 8-287. Similarly, the positions occupied by all the other fragments of sizes between 6 amino acids and 286 amino acids in SEQ ID NO: 4 are included in the present invention and can also be immediately envisaged based on these two examples and therefore, are not individually listed solely for the purpose of not unnecessarily lengthening the specification. Furthermore, the positions occupied by fragments of 6 to 286 consecutive amino acids in SEQ ID NO: 4 are included in the present invention and can also be immediately envisaged based on these two examples and therefore are not individually listed solely for the purpose of not unnecessarily lengthening the specification. In addition, the positions occupied by fragments of 6 consecutive amino acids to 1 amino acid less than any other full-length XCRF3 polypeptide can also be envisaged based on these two examples and therefore are not individually listed solely for the purpose of not unnecessarily lengthening the specification.

The XCRF3 polypeptides of the present invention may alternatively be described by the formula “n to c” (inclusive); where "n" equals the N-terminal most amino acid position (as defined by the sequence listing) and "c" equals the C-terminal most amino acid position (as defined by the sequence listing) of the polypeptide; and further where "n" equals an integer between 1 and the number of amino acids of the full-length polypeptide sequence of the present invention minus 5; and where "c" equals an integer between 6 and the number of amino acids of the full-length polypeptide sequence; and where "n" is an integer smaller then "c" by at least 6. Therefore, for the sequences provided in SEQ ID NO: 4, “n” is any integer selected from the list consisting of: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281 or 282 and “c” is any integer selected from the group consisting of: 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286 or 287. Every combination of “n” and “c” positions are included as specific embodiments of the invention. Moreover, the formula “n” to “c” may

be modified as "'n1 – n2" to "c1 – c2"', wherein "n1 – n2" and "c1 – c2" represent positional ranges selected from any two integers above which represent amino acid positions of the sequence listing. Alternative formulas include "'n1 – n2" to "c"' and "'n" to "c1 – c2"'. In a preferred embodiment, XCRF3 polypeptide fragments of the invention may be described by the formula where n=22 and c=287 of SEQ ID NO: 4.

These specific embodiments, and other polypeptide and polynucleotide fragment embodiments described herein may be modified as being "at least", "equal to", "equal to or less than", "less than", "at least __ but not greater than ___" or "from ___ to ___" a specified size or specified N-terminal and/or C-terminal positions. It is noted that all ranges used to describe any embodiment of the present invention are inclusive unless specifically set forth otherwise.

The present invention also provides for the exclusion of any individual fragment specified by N-terminal and C-terminal positions or of any fragment specified by size in amino acid residues as described above. In addition, any number of fragments specified by N-terminal and C-terminal positions or by size in amino acid residues as described above may be excluded as individual species. Further, any number of fragments specified by N-terminal and C-terminal positions or by size in amino acid residues as described above may make up a polypeptide fragment in any combination and may optionally include non-XCRF3 polypeptide sequences as well.

In preferred embodiments, XCRF3 polypeptide fragments comprising all or part of the globular C-terminal C1q homology domain and having activity selected from the group consisting of lipid partitioning, lipid metabolism, and insulin-like activity are selected from amino acids 22-287, 23-287, 24-287, 25-287, 26-287, 27-287, 28-287, 29-287, 30-287, 31-287, 32-287, 33-287, 34-287, 35-287, 36-287, 37-287, 38-287, 39-287, 40-287, 41-287, 42-287, 43-287, 44-287, 45-287, 46-287, 47-287, 48-287, 49-287, 50-287, 51-287, 52-287, 53-287, 54-287, 55-287, 56-287, 57-287, 58-287, 59-287, 60-287, 61-287, 62-287, 63-287, 64-287, 65-287, 66-287, 67-287, 68-287, 69-287, 70-287, 71-287, 72-287, 73-287, 74-287, 75-287, 76-287, 77-287, 78-287, 79-287, 80-287, 81-287, 82-287, 83-287, 84-287, 85-287, 86-287, 87-287, 88-287, 89-287, 90-287, 91-287, 92-287, 93-287, 94-287, 95-287, 96-287, 97-287, 98-287, 99-287, 100-287, 101-287, 102-287, 103-287, 104-287, 105-287, 106-287, 107-287, 108-287, 109-287, 110-287, 111-287, 112-287, 113-287, 114-287, 115-287, 116-287, 117-287, 118-287, 119-287, 120-287, 121-287, 122-287, 123-287, 124-287, 125-287, 126-287, 127-287, 128-287, 129-287, 130-287, 131-287, 132-287, 133-287, 134-287, 135-287, 136-287, 137-287, 138-287, 139-287, 140-287, 141-287, 142-287, 143-287, 144-287, 145-287, 146-287, 147-287, 148-287, 149-287, 150-287, 151-287, 152-287, 153-287, 154-287, 155-287, 156-287, 157-287, 158-287 or 159-287 of SEQ ID NO: 4, where it is understood that amino acid 22 is taken to represent the N-terminal amino acid of mature XCRF3 polypeptide absent the signal peptide.

In more preferred embodiments, XCRF3 polypeptide fragments comprised of all or part of the globular C-terminal C1q homology domain and having activity selected from the group consisting of lipid partitioning, lipid metabolism, and insulin-like activity are selected from amino acids 22-287, 44-287, 121-287, 122-287, 123-287, 124-287, 125-287, 126-287, 127-287, 128-287, 129-287, 130-287, 131-287, 132-287, 133-287, 134-287, 135-287, 136-287, 137-287, 138-287, 139-287, 140-287, 141-287, 142-287, 143-287, 144-287, 145-287, 146-287, 147-287, 148-287, 149-287, 150-287, 151-287, 152-287, 153-287, 154-287, 155-287, 156-287, 157-287, 158-287 or 159-287 of SEQ ID NO: 4, where it is understood that amino acid 22 is taken to represent the N-terminal amino acid of mature XCRF3 polypeptide absent the signal peptide.

In yet more preferred embodiments, XCRF3 polypeptide fragments comprised of all or part of the globular C-terminal C1q homology domain and having activity are selected from amino acids 22-287, 123-287, 124-287, 125-287, 126-287, 127-287, 128-287, 129-287, 130-287, 131-287, 132-287, 133-287, 134-287, 135-287, 136-287, 137-287, 138-287, 139-287, 140-287, 141-287, 142-287, 143-287, 144-287, 145-287, 146-287, 147-287, 148-287, 149-287, 150-287, 151-287, 152-287 or 153-287 of SEQ ID NO: 4, where it is understood that amino acid 22 is taken to represent the N-terminal amino acid of mature XCRF3 polypeptide absent the signal peptide.

Any XCRF3 polypeptide fragment comprised of all or part of the globular C-terminal C1q homology domain and having activity, as described above, may be excluded.

In other preferred embodiments, said XCRF3 polypeptide fragments comprised of all or part of the globular C-terminal C1q homology domain and having activity selected from the group consisting of prevention of weight gain, weight reduction, and maintenance of weight loss are selected from amino acids 22-287, 23-287, 24-287, 25-287, 26-287, 27-287, 28-287, 29-287, 30-287, 31-287, 32-287, 33-287, 34-287, 35-287, 36-287, 37-287, 38-287, 39-287, 40-287, 41-287, 42-287, 43-287, 44-287, 45-287, 46-287, 47-287, 48-287, 49-287, 50-287, 51-287, 52-287, 53-287, 54-287, 55-287, 56-287, 57-287, 58-287, 59-287, 60-287, 61-287, 62-287, 63-287, 64-287, 65-287, 66-287, 67-287, 68-287, 69-287, 70-287, 71-287, 72-287, 73-287, 74-287, 75-287, 76-287, 77-287, 78-287, 79-287, 80-287, 81-287, 82-287, 83-287, 84-287, 85-287, 86-287, 87-287, 88-287, 89-287, 90-287, 91-287, 92-287, 93-287, 94-287, 95-287, 96-287, 97-287, 98-287, 99-287, 100-287, 101-287, 102-287, 103-287, 104-287, 105-287, 106-287, 107-287, 108-287, 109-287, 110-287, 111-287, 112-287, 113-287, 114-287, 115-287, 116-287, 117-287, 118-287, 119-287, 120-287, 121-287, 122-287, 123-287, 124-287, 125-287, 126-287, 127-287, 128-287, 129-287, 130-287, 131-287, 132-287, 133-287, 134-287, 135-287, 136-287, 137-287, 138-287, 139-287, 140-287, 141-287, 142-287, 143-287, 144-287, 145-287, 146-287, 147-287, 148-287, 149-287, 150-287, 151-287, 152-287, 153-287, 154-287, 155-287, 156-287, 157-287, 158-287 or 159-287 of SEQ

ID NO: 4, where it is understood that amino acid 22 is taken to represent the N-terminal amino acid of mature XCRF3 polypeptide absent the signal peptide.

In other more preferred embodiments, XCRF3 polypeptide fragments comprised of all or part of the globular C-terminal C1q homology domain and having activity selected from the group consisting of prevention of weight gain, weight reduction, and maintenance of weight loss are selected from amino acids 22-287, 44-287, 121-287, 122-287, 123-287, 124-287, 125-287, 126-287, 127-287, 128-287, 129-287, 130-287, 131-287, 132-287, 133-287, 134-287, 135-287, 136-287, 137-287, 138-287, 139-287, 140-287, 141-287, 142-287, 143-287, 144-287, 145-287, 146-287, 147-287, 148-287, 149-287, 150-287, 151-287, 152-287, 153-287, 154-287, 155-287, 156-287, 157-287, 158-287 or 159-287 of SEQ ID NO: 4, where it is understood that amino acid 16 is taken to represent the N-terminal amino acid of mature XCRF3 polypeptide absent the signal peptide.

In other yet more preferred embodiments, XCRF3 polypeptide fragments comprised of all or part of the globular C-terminal C1q homology domain and having activity are selected from amino acids 22-287, 123-287, 124-287, 125-287, 126-287, 127-287, 128-287, 129-287, 130-287, 131-287, 132-287, 133-287, 134-287, 135-287, 136-287, 137-287, 138-287, 139-287, 140-287, 141-287, 142-287, 143-287, 144-287, 145-287, 146-287, 147-287, 148-287, 149-287, 150-287, 151-287, 152-287 or 153-287 of SEQ ID NO: 4, where it is understood that amino acid 16 is taken to represent the N-terminal amino acid of mature XCRF3 polypeptide absent the signal peptide.

Any XCRF3 polypeptide fragment comprised of all or part of the globular C-terminal C1q homology domain and having activity, as described above, may be excluded.

Preferably the XCRF3 polypeptide fragment is mammalian, preferably human or mouse, but most preferably human.

XCRF3 polypeptide fragments of the invention include variants, fragments, analogs and derivatives of the XCRF3 polypeptide fragments described above, including modified XCRF3 polypeptide fragments.

Regulated proteolytic cleavage of full-length XCRF3 polypeptides of the invention *in vivo* is believed by the inventor to lead to the effective generation *in vivo* of XCRF3 polypeptide fragments of the invention comprised of all or part of the globular C-terminal C1q homology domain and having activity selected from the group consisting of lipid partitioning, lipid metabolism, or insulin-like activity. Without wishing to be bound by any particular theory, particularly preferred XCRF3 polypeptide fragments of the invention comprised of all or part of the globular C-terminal C1q homology domain and having activity selected from the group consisting of lipid partitioning, lipid metabolism, and insulin-like activity are fragments of XCRF3 polypeptide of SEQ ID NO: 4 generated by proteolytic cleavage. Without wishing to be bound by any particular theory, other particularly preferred XCRF3

polypeptide fragments of the invention comprised of all or part of the globular C-terminal C1q homology domain and having activity selected from the group consisting of prevention of weight gain, weight reduction, and maintenance of weight loss are fragments of XCRF3 polypeptide of SEQ ID NO: 4 generated by proteolytic cleavage.

XCRF3 polypeptides of the invention include variants, fragments, analogs and derivatives of the XCRF3 polypeptides described above, including modified XCRF3 polypeptides.

Ffor the polypeptide of SEQ ID NO: 6, an XCRF4 polypeptide fragment can be any integer of consecutive amino acids from 6 to 237, for example. The term "integer" is used herein in its mathematical sense and thus representative integers include, but are not limited to: 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236 and 237.

Each XCRF4 polypeptide fragment as described above can be further specified in terms of its N-terminal and C-terminal positions. For example, every combination of a N-terminal and C-terminal position that fragments of from 6 contiguous amino acids to one amino acid less than the full-length polypeptide of SEQ ID NO: 6 could occupy, on any given intact and contiguous full-length polypeptide sequence of SEQ ID NO: 6 are included in the present invention. Thus, a 6 consecutive amino acid fragment could occupy positions selected from the group consisting of 1-6, 2-7, 3-8, 4-9, 5-10, 6-11, 7-12, 8-13, 9-14, 10-15, 11-16, 12-17, 13-18, 14-19, 15-20, 16-21, 17-22, 18-23, 19-24, 20-25, 21-26, 22-27, 23-28, 24-29, 25-30, 26-31, 27-32, 28-33, 29-34, 30-35, 31-36, 32-37, 33-38, 34-39, 35-40, 36-41, 37-42, 38-43, 39-44, 40-45, 41-46, 42-47, 43-48, 44-49, 45-50, 46-51, 47-52, 48-53, 49-54, 50-55, 51-56, 52-57, 53-58, 54-59, 55-60, 56-61, 57-62, 58-63, 59-64, 60-65, 61-66, 62-67, 63-68, 64-69, 65-70, 66-71, 67-72, 68-73, 69-74, 70-75, 71-76, 72-77, 73-78, 74-79, 75-80, 76-81, 77-82, 78-83, 79-84, 80-85, 81-86, 82-87, 83-88, 84-89, 85-90, 86-91, 87-92, 88-93, 89-94, 90-95, 91-96, 92-97, 93-98, 94-99, 95-100, 96-101, 97-102, 98-103, 99-104, 100-105, 101-106, 102-107, 103-108, 104-109, 105-110, 106-111, 107-112, 108-113, 109-114, 110-115, 111-116, 112-117, 113-118, 114-119, 115-120, 116-121, 117-122, 118-123, 119-124, 120-125, 121-126, 122-127, 123-128, 124-129, 125-130, 126-131, 127-132,

128-133, 129-134, 130-135, 131-136, 132-137, 133-138, 134-139, 135-140, 136-141, 137-142, 138-143, 139-144, 140-145, 141-146, 142-147, 143-148, 144-149, 145-150, 146-151, 147-152, 148-153, 149-154, 150-155, 151-156, 152-157, 153-158, 154-159, 155-160, 156-161, 157-162, 158-163, 159-164, 160-165, 161-166, 162-167, 163-168, 164-169, 165-170, 166-171, 167-172, 168-173, 169-174, 170-175, 171-176, 172-177, 173-178, 174-179, 175-180, 176-181, 177-182, 178-183, 179-184, 180-185, 181-186, 182-187, 183-188, 184-189, 185-190, 186-191, 187-192, 188-193, 189-194, 190-195, 191-196, 192-197, 193-198, 194-199, 195-200, 196-201, 197-202, 198-203, 199-204, 200-205, 201-206, 202-207, 203-208, 204-209, 205-210, 206-211, 207-212, 208-213, 209-214, 210-215, 211-216, 212-217, 213-218, 214-219, 215-220, 216-221, 217-222, 218-223, 219-224, 220-225, 221-226, 222-227, 223-228, 224-229, 225-230, 226-231, 227-232, 228-233, 229-234, 230-235, 231-236, 232-237 and 233-238 of a 238 consecutive amino acid fragment. A 230 consecutive amino acid fragment could occupy positions selected from the group consisting of 1-230, 2-231, 3-232, 4-233, 5-234, 6-235, 7-236, 8-237 and 9-238. Similarly, the positions occupied by all the other fragments of sizes between 6 amino acids and 237 amino acids in SEQ ID NO: 6 are included in the present invention and can also be immediately envisaged based on these two examples and therefore, are not individually listed solely for the purpose of not unnecessarily lengthening the specification. Furthermore, the positions occupied by fragments of 6 to 237 consecutive amino acids in SEQ ID NO: 6 are included in the present invention and can also be immediately envisaged based on these two examples and therefore are not individually listed solely for the purpose of not unnecessarily lengthening the specification. In addition, the positions occupied by fragments of 6 consecutive amino acids to 1 amino acid less than any other full-length XCRF4 polypeptide can also be envisaged based on these two examples and therefore are not individually listed solely for the purpose of not unnecessarily lengthening the specification.

The XCRF4 polypeptides of the present invention may alternatively be described by the formula "n to c" (inclusive); where "n" equals the N-terminal most amino acid position (as defined by the sequence listing) and "c" equals the C-terminal most amino acid position (as defined by the sequence listing) of the polypeptide; and further where "n" equals an integer between 1 and the number of amino acids of the full-length polypeptide sequence of the present invention minus 5; and where "c" equals an integer between 6 and the number of amino acids of the full-length polypeptide sequence; and where "n" is an integer smaller then "c" by at least 6. Therefore, for the sequences provided in SEQ ID NO: 6, "n" is any integer selected from the list consisting of: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121,

122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232 or 233 and "c" is any integer selected from the group consisting of: 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237 or 238. Every combination of "n" and "c" positions are included as specific embodiments of the invention. Moreover, the formula "n" to "c" may be modified as '"n1 – n2" to "c1 – c2"', wherein "n1 – n2" and "c1 – c2" represent positional ranges selected from any two integers above which represent amino acid positions of the sequence listing. Alternative formulas include '"n1 – n2" to "c"' and '"n" to "c1 – c2"'. In a preferred embodiment, XCRF4 polypeptide fragments of the invention may be described by the formula where n1=16, n2=110, and c=238 of SEQ ID NO: 6.

In preferred embodiments, XCRF4 polypeptide fragments having activity are selected from amino acids 16-238, 17-238, 18-238, 19-238, 20-238, 21-238, 22-238, 23-238, 24-238, 25-238, 26-238, 27-238, 28-238, 29-238, 30-238, 31-238, 32-238, 33-238, 34-238, 35-238, 36-238, 37-238, 38-238, 39-238, 40-238, 41-238, 42-238, 43-238, 44-238, 45-238, 46-238, 47-238, 48-238, 49-238, 50-238, 51-238, 52-238, 53-238, 54-238, 55-238, 56-238, 57-238, 58-238, 59-238, 60-238, 61-238, 62-238, 63-238, 64-238, 65-238, 66-238, 67-238, 68-238, 69-238, 70-238, 71-238, 72-238, 73-238, 74-238, 75-238, 76-238, 77-238, 78-238, 79-238, 80-238, 81-238, 82-238, 83-238, 84-238, 85-238, 86-238, 87-238, 88-238, 89-238, 90-238, 91-238, 92-238, 93-238, 94-238, 95-238, 96-238, 97-238, 98-238, 99-238, 100-238, 101-238, 102-238, 103-238, 104-238, 105-238, 106-238, 107-238, 108-238, 109-238 or 110-238 of SEQ ID NO: 6, where it is understood that amino acid 16 is taken to represent the N-terminal amino acid of mature XCRF4 polypeptide absent the signal peptide.

These specific embodiments, and other polypeptide and polynucleotide fragment embodiments described herein may be modified as being "at least", "equal to", "equal to or less than", "less than", "at least __ but not greater than ___" or "from ___ to ___" a specified size or specified N-terminal and/or C-terminal positions. It is noted that all ranges used to describe any embodiment of the present invention are inclusive unless specifically set forth otherwise.

The present invention also provides for the exclusion of any individual fragment specified by N-terminal and C-terminal positions or of any fragment specified by size in amino acid residues as described above. In addition, any number of fragments specified by N-terminal and C-terminal positions or by size in amino acid residues as described above may be excluded as individual species. Further, any number of fragments specified by N-terminal and C-terminal positions or by size in amino acid residues as described above may make up a polypeptide fragment in any combination and may optionally include non-XCRF4 polypeptide sequences as well.

In preferred embodiments, XCRF4 polypeptide fragments comprising all or part of the globular C-terminal C1q homology domain and having activity selected from the group consisting of lipid partitioning, lipid metabolism, and insulin-like activity are selected from amino acids 16-238, 17-238, 18-238, 19-238, 20-238, 21-238, 22-238, 23-238, 24-238, 25-238, 26-238, 27-238, 28-238, 29-238, 30-238, 31-238, 32-238, 33-238, 34-238, 35-238, 36-238, 37-238, 38-238, 39-238, 40-238, 41-238, 42-238, 43-238, 44-238, 45-238, 46-238, 47-238, 48-238, 49-238, 50-238, 51-238, 52-238, 53-238, 54-238, 55-238, 56-238, 57-238, 58-238, 59-238, 60-238, 61-238, 62-238, 63-238, 64-238, 65-238, 66-238, 67-238, 68-238, 69-238, 70-238, 71-238, 72-238, 73-238, 74-238, 75-238, 76-238, 77-238, 78-238, 79-238, 80-238, 81-238, 82-238, 83-238, 84-238, 85-238, 86-238, 87-238, 88-238, 89-238, 90-238, 91-238, 92-238, 93-238, 94-238, 95-238, 96-238, 97-238, 98-238, 99-238, 100-238, 101-238, 102-238, 103-238, 104-238, 105-238, 106-238, 107-238, 108-238, 109-238 or 110-238 of SEQ ID NO: 6, where it is understood that amino acid 16 is taken to represent the N-terminal amino acid of mature XCRF4 polypeptide absent the signal peptide.

In more preferred embodiments, XCRF4 polypeptide fragments comprised of all or part of the globular C-terminal C1q homology domain and having activity selected from the group consisting of lipid partitioning, lipid metabolism, and insulin-like activity are selected from amino acids 16-238, 18-238, 28-238, 30-238, 39-238, 58-238, 63-238, 64-238, 66-238, 70-238, 79-238, 87-238, 97-238, 98-238, 99-238, 100-238, 101-238, 102-238, 103-238, 104-238, 105-238, 106-238, 107-238, 108-238, 109-238 or 110-238 of SEQ ID NO: 6, where it is understood that amino acid 16 is taken to represent the N-terminal amino acid of mature XCRF4 polypeptide absent the signal peptide.

In yet more preferred embodiments, XCRF4 polypeptide fragments comprised of all or part of the globular C-terminal C1q homology domain and having activity are selected from amino acids 16-

238, 97-238, 98-238, 99-238, 100-238, 101-238, 102-238, 103-238, 104-238, 105-238, 106-238, 107-238, 108-238, 109-238 or 110-238 of SEQ ID NO: 6, where it is understood that amino acid 16 is taken to represent the N-terminal amino acid of mature XCRF4 polypeptide absent the signal peptide.

In other preferred embodiments, said XCRF4 polypeptide fragments comprised of all or part of the globular C-terminal C1q homology domain and having activity selected from the group consisting of prevention of weight gain, weight reduction, and maintenance of weight loss are selected from amino acids 16-238, 17-238, 18-238, 19-238, 20-238, 21-238, 22-238, 23-238, 24-238, 25-238, 26-238, 27-238, 28-238, 29-238, 30-238, 31-238, 32-238, 33-238, 34-238, 35-238, 36-238, 37-238, 38-238, 39-238, 40-238, 41-238, 42-238, 43-238, 44-238, 45-238, 46-238, 47-238, 48-238, 49-238, 50-238, 51-238, 52-238, 53-238, 54-238, 55-238, 56-238, 57-238, 58-238, 59-238, 60-238, 61-238, 62-238, 63-238, 64-238, 65-238, 66-238, 67-238, 68-238, 69-238, 70-238, 71-238, 72-238, 73-238, 74-238, 75-238, 76-238, 77-238, 78-238, 79-238, 80-238, 81-238, 82-238, 83-238, 84-238, 85-238, 86-238, 87-238, 88-238, 89-238, 90-238, 91-238, 92-238, 93-238, 94-238, 95-238, 96-238, 97-238, 98-238, 99-238, 100-238, 101-238, 102-238, 103-238, 104-238, 105-238, 106-238, 107-238, 108-238, 109-238 or 110-238 of SEQ ID NO: 6, where it is understood that amino acid 16 is taken to represent the N-terminal amino acid of mature XCRF4 polypeptide absent the signal peptide.

In other more preferred embodiments, XCRF4 polypeptide fragments comprised of all or part of the globular C-terminal C1q homology domain and having activity selected from the group consisting of prevention of weight gain, weight reduction, and maintenance of weight loss are selected from amino acids 16-238, 18-238, 28-238, 30-238, 39-238, 58-238, 63-238, 64-238, 66-238, 70-238, 79-238, 87-238, 97-238, 98-238, 99-238, 100-238, 101-238, 102-238, 103-238, 104-238, 105-238, 106-238, 107-238, 108-238, 109-238 or 110-238 of SEQ ID NO: 6, where it is understood that amino acid 16 is taken to represent the N-terminal amino acid of mature XCRF4 polypeptide absent the signal peptide.

In other yet more preferred embodiments, XCRF4 polypeptide fragments comprised of all or part of the globular C-terminal C1q homology domain and having activity are selected from amino acids 16-238, 97-238, 98-238, 99-238, 100-238, 101-238, 102-238, 103-238, 104-238, 105-238, 106-238, 107-238, 108-238, 109-238 or 110-238 of SEQ ID NO: 6, where it is understood that amino acid 16 is taken to represent the N-terminal amino acid of mature XCRF4 polypeptide absent the signal peptide.

Preferably the XCRF4 polypeptide fragment is mammalian, preferably human or mouse, but most preferably human.

XCRF4 polypeptide fragments of the invention include variants, fragments, analogs and derivatives of the XCRF4 polypeptide fragments described above, including modified XCRF4 polypeptide fragments.

Regulated proteolytic cleavage of full-length XCRF4 polypeptides of the invention *in vivo* is believed by the inventors to lead to the effective generation *in vivo* of XCRF4 polypeptide fragments of the invention comprised of all or part of the globular C-terminal C1q homology domain and having activity selected from the group consisting of lipid partitioning, lipid metabolism, or insulin-like activity. Without wishing to be bound by any particular theory, particularly preferred XCRF4 polypeptide fragments of the invention comprised of all or part of the globular C-terminal C1q homology domain and having activity selected from the group consisting of lipid partitioning, lipid metabolism, and insulin-like activity are fragments of XCRF4 polypeptide of SEQ ID NO: 6 generated by proteolytic cleavage. Without wishing to be bound by any particular theory, other particularly preferred XCRF4 polypeptide fragments of the invention comprised of all or part of the globular C-terminal C1q homology domain and having activity selected from the group consisting of prevention of weight gain, weight reduction, and maintenance of weight loss are fragments of XCRF4 polypeptide of SEQ ID NO: 6 generated by proteolytic cleavage.

Without wishing to be bound by any particular theory, also particularly preferred are XCRF4 polypeptide fragments of amino acids 18-238, 28-238, 30-238, 39-238, 58-238, 63-238, 64-238, 66-238, 70-238, 79-238, 87-238 or 110-238 of SEQ ID NO:6 generated by plasmin cleavage.

Without wishing to be bound by any particular theory, also particularly preferred are XCRF4 polypeptide fragments of amino acids 18-238, 28-238, 30-238, 39-238, 58-238, 63-238, 64-238, 66-238, 70-238, 79-238, 87-238 or 110-238 of SEQ ID NO:6 generated by trypsin cleavage.

XCRF4 polypeptides of the invention include variants, fragments, analogs and derivatives of the XCRF4 polypeptides described above, including modified XCRF4 polypeptides.

For the polypeptide of SEQ ID NO: 8, an XCRF5 polypeptide fragment can be any integer of consecutive amino acids from 6 to 286, for example. The term "integer" is used herein in its mathematical sense and thus representative integers include, but are not limited to: 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237 238, 239, 240, 241, 242, 243,

244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285 and 286.

Each XCRF5 polypeptide fragment as described above can be further specified in terms of its N-terminal and C-terminal positions. For example, every combination of a N-terminal and C-terminal position that fragments of from 6 contiguous amino acids to one amino acid less than the full-length polypeptide of SEQ ID NO: 8 could occupy, on any given intact and contiguous full-length polypeptide sequence of SEQ ID NO: 8 are included in the present invention. Thus, a 6 consecutive amino acid fragment could occupy positions selected from the group consisting of 1-6, 2-7, 3-8, 4-9, 5-10, 6-11, 7-12, 8-13, 9-14, 10-15, 11-16, 12-17, 13-18, 14-19, 15-20, 16-21, 17-22, 18-23, 19-24, 20-25, 21-26, 22-27, 23-28, 24-29, 25-30, 26-31, 27-32, 28-33, 29-34, 30-35, 31-36, 32-37, 33-38, 34-39, 35-40, 36-41, 37-42, 38-43, 39-44, 40-45, 41-46, 42-47, 43-48, 44-49, 45-50, 46-51, 47-52, 48-53, 49-54, 50-55, 51-56, 52-57, 53-58, 54-59, 55-60, 56-61, 57-62, 58-63, 59-64, 60-65, 61-66, 62-67, 63-68, 64-69, 65-70, 66-71, 67-72, 68-73, 69-74, 70-75, 71-76, 72-77, 73-78, 74-79, 75-80, 76-81, 77-82, 78-83, 79-84, 80-85, 81-86, 82-87, 83-88, 84-89, 85-90, 86-91, 87-92, 88-93, 89-94, 90-95, 91-96, 92-97, 93-98, 94-99, 95-100, 96-101, 97-102, 98-103, 99-104, 100-105, 101-106, 102-107, 103-108, 104-109, 105-110, 106-111, 107-112, 108-113, 109-114, 110-115, 111-116, 112-117, 113-118, 114-119, 115-120, 116-121, 117-122, 118-123, 119-124, 120-125, 121-126, 122-127, 123-128, 124-129, 125-130, 126-131, 127-132, 128-133, 129-134, 130-135, 131-136, 132-137, 133-138, 134-139, 135-140, 136-141, 137-142, 138-143, 139-144, 140-145, 141-146, 142-147, 143-148, 144-149, 145-150, 146-151, 147-152, 148-153, 149-154, 150-155, 151-156, 152-157, 153-158, 154-159, 155-160, 156-161, 157-162, 158-163, 159-164, 160-165, 161-166, 162-167, 163-168, 164-169, 165-170, 166-171, 167-172, 168-173, 169-174, 170-175, 171-176, 172-177, 173-178, 174-179, 175-180, 176-181, 177-182, 178-183, 179-184, 180-185, 181-186, 182-187, 183-188, 184-189, 185-190, 186-191, 187-192, 188-193, 189-194, 190-195, 191-196, 192-197, 193-198, 194-199, 195-200, 196-201, 197-202, 198-203, 199-204, 200-205, 201-206, 202-207, 203-208, 204-209, 205-210, 206-211, 207-212, 208-213, 209-214, 210-215, 211-216, 212-217, 213-218, 214-219, 215-220, 216-221, 217-222, 218-223, 219-224, 220-225, 221-226, 222-227, 223-228, 224-229, 225-230, 226-231, 227-232, 228-233, 229-234, 230-235, 231-236, 232-237, 233-238, 234-239, 235-240, 236-241, 237-242, 238-243, 239-244, 240-245, 241-246, 242-247, 243-248, 244-249, 245-250, 246-251, 247-252, 248-253, 249-254, 250-255, 251-256, 252-257, 253-258, 254-259, 255-260, 256-261, 257-262, 258-263, 259-264, 260-265, 261-266, 262-267, 263-268, 264-269, 265-270, 266-271, 267-272, 268-273, 269-274, 270-275, 271-276, 272-277, 273-278, 274-279, 275-280, 276-281, 277-282, 278-283, 279-284, 280-285, 281-286 and 282-287 of a 287 consecutive amino acid polypeptide. A 280 consecutive amino acid fragment could occupy positions selected from the group consisting of 1-280, 2-281, 3-282, 4-283, 5-284, 6-285,

7-286, and 8-287. Similarly, the positions occupied by all the other fragments of sizes between 6 amino acids and 286 amino acids in SEQ ID NO: 8 are included in the present invention and can also be immediately envisaged based on these two examples and therefore, are not individually listed solely for the purpose of not unnecessarily lengthening the specification. Furthermore, the positions occupied by fragments of 6 to 286 consecutive amino acids in SEQ ID NO: 8 are included in the present invention and can also be immediately envisaged based on these two examples and therefore are not individually listed solely for the purpose of not unnecessarily lengthening the specification. In addition, the positions occupied by fragments of 6 consecutive amino acids to 1 amino acid less than any other full-length XCRF5 polypeptide can also be envisaged based on these two examples and therefore are not individually listed solely for the purpose of not unnecessarily lengthening the specification.

The XCRF5 polypeptides of the present invention may alternatively be described by the formula "n to c" (inclusive); where "n" equals the N-terminal most amino acid position (as defined by the sequence listing) and "c" equals the C-terminal most amino acid position (as defined by the sequence listing) of the polypeptide; and further where "n" equals an integer between 1 and the number of amino acids of the full-length polypeptide sequence of the present invention minus 5; and where "c" equals an integer between 6 and the number of amino acids of the full-length polypeptide sequence; and where "n" is an integer smaller then "c" by at least 6. Therefore, for the sequences provided in SEQ ID NO: 8, "n" is any integer selected from the list consisting of: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281 or 282 and "c" is any integer selected from the group consisting of: 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107,

108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286 or 287. Every combination of "n" and "c" positions are included as specific embodiments of the invention. Moreover, the formula "n" to "c" may be modified as '"n1 – n2" to "c1 – c2"', wherein "n1 – n2" and "c1 – c2" represent positional ranges selected from any two integers above which represent amino acid positions of the sequence listing. Alternative formulas include '"n1 – n2" to "c"' and '"n" to "c1 – c2"'. In a preferred embodiment, XCRF5 polypeptide fragments of the invention may be described by the formula where n=22 and c=287 of SEQ ID NO: 8. In other preferred embodiment, XCRF5 polypeptide fragments of the invention may be described by the formula where n1=121, n2=159, and c=287 of SEQ ID NO: 8.

In preferred embodiments, XCRF5 polypeptide fragments having activity are selected from amino acids 22-287, 121-287, 122-287, 123-287, 124-287, 125-287, 126-287, 127-287, 128-287, 129-287, 130-287, 131-287, 132-287, 133-287, 134-287, 135-287, 136-287, 137-287, 138-287, 139-287, 140-287, 141-287, 142-287, 143-287, 144-287, 145-287, 146-287, 147-287, 148-287, 149-287, 150-287, 151-287, 152-287, 153-287, 154-287, 155-287, 156-287, 157-287, 158-287 or 159-287 of SEQ ID NO: 8, where it is understood that amino acid 22 is taken to represent the N-terminal amino acid of mature XCRF5 polypeptide absent the signal peptide. Any said XCRF5 polypeptide fragment having activity may be excluded.

These specific embodiments, and other polypeptide and polynucleotide fragment embodiments described herein may be modified as being "at least", "equal to", "equal to or less than", "less than", "at least __ but not greater than ___" or "from ___ to ___" a specified size or specified N-terminal and/or C-terminal positions. It is noted that all ranges used to describe any embodiment of the present invention are inclusive unless specifically set forth otherwise.

The present invention also provides for the exclusion of any individual fragment specified by N-terminal and C-terminal positions or of any fragment specified by size in amino acid residues as described above. In addition, any number of fragments specified by N-terminal and C-terminal positions or by size in amino acid residues as described above may be excluded as individual species. Further, any number of fragments specified by N-terminal and C-terminal positions or by size in amino acid

residues as described above may make up a polypeptide fragment in any combination and may optionally include non-XCRF5 polypeptide sequences as well.

In preferred embodiments, XCRF5 polypeptide fragments comprising all or part of the globular C-terminal C1q homology domain and having activity selected from the group consisting of lipid partitioning, lipid metabolism, and insulin-like activity are selected from amino acids 22-287, 23-287, 24-287, 25-287, 26-287, 27-287, 28-287, 29-287, 30-287, 31-287, 32-287, 33-287, 34-287, 35-287, 36-287, 37-287, 38-287, 39-287, 40-287, 41-287, 42-287, 43-287, 44-287, 45-287, 46-287, 47-287, 48-287, 49-287, 50-287, 51-287, 52-287, 53-287, 54-287, 55-287, 56-287, 57-287, 58-287, 59-287, 60-287, 61-287, 62-287, 63-287, 64-287, 65-287, 66-287, 67-287, 68-287, 69-287, 70-287, 71-287, 72-287, 73-287, 74-287, 75-287, 76-287, 77-287, 78-287, 79-287, 80-287, 81-287, 82-287, 83-287, 84-287, 85-287, 86-287, 87-287, 88-287, 89-287, 90-287, 91-287, 92-287, 93-287, 94-287, 95-287, 96-287, 97-287, 98-287, 99-287, 100-287, 101-287, 102-287, 103-287, 104-287, 105-287, 106-287, 107-287, 108-287, 109-287, 110-287, 111-287, 112-287, 113-287, 114-287, 115-287, 116-287, 117-287, 118-287, 119-287, 120-287, 121-287, 122-287, 123-287, 124-287, 125-287, 126-287, 127-287, 128-287, 129-287, 130-287, 131-287, 132-287, 133-287, 134-287, 135-287, 136-287, 137-287, 138-287, 139-287, 140-287, 141-287, 142-287, 143-287, 144-287, 145-287, 146-287, 147-287, 148-287, 149-287, 150-287, 151-287, 152-287, 153-287, 154-287, 155-287, 156-287, 157-287, 158-287 or 159-287 of SEQ ID NO: 8, where it is understood that amino acid 22 is taken to represent the N-terminal amino acid of mature XCRF5 polypeptide absent the signal peptide.

In more preferred embodiments, XCRF5 polypeptide fragments comprised of all or part of the globular C-terminal C1q homology domain and having activity selected from the group consisting of lipid partitioning, lipid metabolism, and insulin-like activity are selected from amino acids 22-287, 121-287, 122-287, 123-287, 124-287, 125-287, 126-287, 127-287, 128-287, 129-287, 130-287, 131-287, 132-287, 133-287, 134-287, 135-287, 136-287, 137-287, 138-287, 139-287, 140-287, 141-287, 142-287, 143-287, 144-287, 145-287, 146-287, 147-287, 148-287, 149-287, 150-287, 151-287, 152-287, 153-287, 154-287, 155-287, 156-287, 157-287, 158-287 or 159-287 of SEQ ID NO: 8, where it is understood that amino acid 22 is taken to represent the N-terminal amino acid of mature XCRF5 polypeptide absent the signal peptide.

In yet more preferred embodiments, XCRF5 polypeptide fragments comprised of all or part of the globular C-terminal C1q homology domain and having activity are selected from amino acids 22-287, 123-287, 124-287, 125-287, 126-287, 127-287, 128-287, 129-287, 130-287, 131-287, 132-287, 133-287, 134-287, 135-287, 136-287, 137-287, 138-287, 139-287, 140-287, 141-287, 142-287, 143-287, 144-287, 145-287, 146-287, 147-287, 148-287, 149-287, 150-287, 151-287, 152-287 or 153-287 of SEQ

ID NO: 8, where it is understood that amino acid 22 is taken to represent the N-terminal amino acid of mature XCRF5 polypeptide absent the signal peptide.

In other preferred embodiments, said XCRF5 polypeptide fragments comprised of all or part of the globular C-terminal C1q homology domain and having activity selected from the group consisting of prevention of weight gain, weight reduction, and maintenance of weight loss are selected from amino acids 22-287, 23-287, 24-287, 25-287, 26-287, 27-287, 28-287, 29-287, 30-287, 31-287, 32-287, 33-287, 34-287, 35-287, 36-287, 37-287, 38-287, 39-287, 40-287, 41-287, 42-287, 43-287, 44-287, 45-287, 46-287, 47-287, 48-287, 49-287, 50-287, 51-287, 52-287, 53-287, 54-287, 55-287, 56-287, 57-287, 58-287, 59-287, 60-287, 61-287, 62-287, 63-287, 64-287, 65-287, 66-287, 67-287, 68-287, 69-287, 70-287, 71-287, 72-287, 73-287, 74-287, 75-287, 76-287, 77-287, 78-287, 79-287, 80-287, 81-287, 82-287, 83-287, 84-287, 85-287, 86-287, 87-287, 88-287, 89-287, 90-287, 91-287, 92-287, 93-287, 94-287, 95-287, 96-287, 97-287, 98-287, 99-287, 100-287, 101-287, 102-287, 103-287, 104-287, 105-287, 106-287, 107-287, 108-287, 109-287, 110-287, 111-287, 112-287, 113-287, 114-287, 115-287, 116-287, 117-287, 118-287, 119-287, 120-287, 121-287, 122-287, 123-287, 124-287, 125-287, 126-287, 127-287, 128-287, 129-287, 130-287, 131-287, 132-287, 133-287, 134-287, 135-287, 136-287, 137-287, 138-287, 139-287, 140-287, 141-287, 142-287, 143-287, 144-287, 145-287, 146-287, 147-287, 148-287, 149-287, 150-287, 151-287, 152-287, 153-287, 154-287, 155-287, 156-287, 157-287, 158-287 or 159-287 of SEQ ID NO: 8, where it is understood that amino acid 16 is taken to represent the N-terminal amino acid of mature XCRF5 polypeptide absent the signal peptide.

In other more preferred embodiments, XCRF5 polypeptide fragments comprised of all or part of the globular C-terminal C1q homology domain and having activity selected from the group consisting of prevention of weight gain, weight reduction, and maintenance of weight loss are selected from amino acids 22-287, 121-287, 122-287, 123-287, 124-287, 125-287, 126-287, 127-287, 128-287, 129-287, 130-287, 131-287, 132-287, 133-287, 134-287, 135-287, 136-287, 137-287, 138-287, 139-287, 140-287, 141-287, 142-287, 143-287, 144-287, 145-287, 146-287, 147-287, 148-287, 149-287, 150-287, 151-287, 152-287, 153-287, 154-287, 155-287, 156-287, 157-287, 158-287 or 159-287 of SEQ ID NO: 8, where it is understood that amino acid 16 is taken to represent the N-terminal amino acid of mature XCRF5 polypeptide absent the signal peptide.

In other yet more preferred embodiments, XCRF5 polypeptide fragments comprised of all or part of the globular C-terminal C1q homology domain and having activity are selected from amino acids 22-287, 123-287, 124-287, 125-287, 126-287, 127-287, 128-287, 129-287, 130-287, 131-287, 132-287, 133-287, 134-287, 135-287, 136-287, 137-287, 138-287, 139-287, 140-287, 141-287, 142-287, 143-287, 144-287, 145-287, 146-287, 147-287, 148-287, 149-287, 150-287, 151-287, 152-287 or 153-287 of SEQ

ID NO: 8, where it is understood that amino acid 16 is taken to represent the N-terminal amino acid of mature XCRF5 polypeptide absent the signal peptide.

Any XCRF5 polypeptide fragment comprised of all or part of the globular C-terminal C1q homology domain and having activity, as described above, may be excluded.

Preferably the XCRF5 polypeptide fragment is mammalian, preferably human or mouse, but most preferably human.

XCRF5 polypeptide fragments of the invention include variants, fragments, analogs and derivatives of the XCRF5 polypeptide fragments described above, including modified XCRF5 polypeptide fragments.

Regulated proteolytic cleavage of full-length XCRF5 polypeptides of the invention *in vivo* is believed by the inventors to lead to the effective generation *in vivo* of XCRF5 polypeptide fragments of the invention comprised of all or part of the globular C-terminal C1q homology domain and having activity selected from the group consisting of lipid partitioning, lipid metabolism, or insulin-like activity. Without wishing to be bound by any particular theory, particularly preferred XCRF5 polypeptide fragments of the invention comprised of all or part of the globular C-terminal C1q homology domain and having activity selected from the group consisting of lipid partitioning, lipid metabolism, and insulin-like activity are fragments of XCRF5 polypeptide of SEQ ID NO: 8 generated by proteolytic cleavage. Without wishing to be bound by any particular theory, other particularly preferred XCRF5 polypeptide fragments of the invention comprised of all or part of the globular C-terminal C1q homology domain and having activity selected from the group consisting of prevention of weight gain, weight reduction, and maintenance of weight loss are fragments of XCRF5 polypeptide of SEQ ID NO: 8 generated by proteolytic cleavage.

Without wishing to be bound by any particular theory, also particularly preferred are XCRF5 polypeptide fragments selected from amino acids 76-287, 77-287, 78-287, 79-287, 80-287, 81-287, 82-287, 83-287, 84-287, 85-287, 86-287, 87-287, 88-287, 89-287, 90-287, 91-287, 92-287, 93-287, 94-287, 95-287, 96-287, 97-287, 98-287, 99-287, 100-287, 101-287, 102-287, 103-287, 104-287, 105-287, 106-287, 107-287, 108-287, 109-287, 110-287, 111-287, 112-287, 113-287, 114-287, 115-287, 116-287, 117-287, 118-287, 119-287 or 120-287 of SEQ ID NO: 8 generated by collagenase cleavage.

Without wishing to be bound by any particular theory, also particularly preferred are XCRF5 polypeptide fragments selected from amino acids 54-287 or 157-287 of SEQ ID NO: 8 generated by matrix metalloproteinase-1 (MMP-1) cleavage.

Without wishing to be bound by any particular theory, also particularly preferred are XCRF5 polypeptide fragments selected from amino acids 31-287, 49-287, 79-287, 84-287, 87-287, 91-287, 103-287, 109-287, 114-287 or 159-287 of SEQ ID NO:8 generated by plasmin cleavage.

Without wishing to be bound by any particular theory, also particularly preferred are XCRF5 polypeptide fragments selected from amino acids 31-287, 49-287, 79-287, 84-287, 87-287, 91-287, 103-287, 109-287, 114-287 or 159-287 of SEQ ID NO:8 generated by trypsin cleavage.
XCRF5 polypeptides of the invention include variants, fragments, analogs and derivatives of the XCRF5 polypeptides described above, including modified XCRF5 polypeptides.

Variants

It will be recognized by one of ordinary skill in the art that some amino acids of the XCRF polypeptide sequences of the present invention can be varied without significant effect on the structure or function of the proteins; there will be critical amino acids in the sequence that determine activity. Thus, the invention further includes variants of XCRF polypeptides that have metabolic-related activity as described above. Such variants include XCRF polypeptide sequences with one or more amino acid deletions, insertions, inversions, repeats, and substitutions either from natural mutations or human manipulation selected according to general rules known in the art so as to have little effect on activity. Guidance concerning how to make phenotypically silent amino acid substitutions is provided below.

There are two main approaches for studying the tolerance of an amino acid sequence to change (*see*, Bowie, et al. (1990) Science, 247, 1306-10). The first method relies on the process of evolution, in which mutations are either accepted or rejected by natural selection. The second approach uses genetic engineering to introduce amino acid changes at specific positions of a cloned gene and selections or screens to identify sequences that maintain functionality.

These studies have revealed that proteins are surprisingly tolerant of amino acid substitutions and indicate which amino acid changes are likely to be permissive at a certain position of the protein. For example, most buried amino acid residues require nonpolar side chains, whereas few features of surface side chains are generally conserved. Other such phenotypically silent substitutions are described by Bowie et al. (*supra*) and the references cited therein.

In the case of an amino acid substitution in the amino acid sequence of a polypeptide according to the invention, one or several amino acids can be replaced by "equivalent" amino acids. The expression “equivalent” amino acid is used herein to designate any amino acid that may be substituted for one of the amino acids having similar properties, such that one skilled in the art of peptide chemistry would expect the secondary structure and hydropathic nature of the polypeptide to be substantially unchanged.

In particular embodiments, conservative substitutions of interest are shown in Table 1 under the heading of preferred substitutions. If such substitutions result in a change in biological activity, then

more substantial changes, denominated exemplary substitutions in Table 1, or as further described below in reference to amino acid classes, are introduced and the products screened.

Table 1

Original Residue	Exemplary Substitutions	Preferred Substitutions
Ala (A)	val; leu; ile	val
Arg (R)	lys; gln; asn	lys
Asn (N)	gln; his; lys; arg	gln
Asp (D)	glu	glu
Cys (C)	ser	ser
Gln (Q)	asn	asn
Glu (E)	asp	asp
Gly (G)	pro; ala	ala
His (H)	asn; gln; lys; arg	arg
Ile (I)	leu; val; met; ala; phe; norleucine	leu
Leu (L)	norleucine; ile; val; met; ala; phe	ile
Lys (K)	arg; gln; asn	arg
Met (M)	leu; phe; ile	leu
Phe (F)	leu; val; ile; ala; tyr	leu
Pro (P)	ala	ala
Ser (S)	thr	thr
Thr (T)	ser	ser
Trp (W)	tyr; phe	tyr
Tyr (Y)	trp; phe; thr; ser	phe
Val (V)	ile; leu; met; phe;ala; norleucine	leu

Substantial modifications in function or immunological identity of the XCRF polypeptides are accomplished by selecting substitutions that differ significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. Naturally occurring residues are divided into groups based on common side-chain properties:

(1) hydrophobic: norleucine, met, ala, val, leu, ile;

(2) neutral hydrophilic: cys, ser, thr;

(3) acidic: asp, glu;

(4) basic: asn, gln, his, lys, arg;

(5) residues that influence chain orientation: gly, pro; and

(6) aromatic: trp, tyr, phe.

Non-conservative substitutions will entail exchanging a member of one of these classes for another class. Such substituted residues also may be introduced into the conservative substitution sites or, more preferably, into the remaining (non-conserved) sites.

The variations can be made using methods known in the art such as oligonucleotide-mediated (site-directed) mutagenesis, alanine scanning, and PCR mutagenesis. Site-directed mutagenesis [Carter *et al.*, Nucl Acids Res, 13:4331 (1986); Zoller *et al.*, Nucl Acids Res, 10:6487 (1987)], cassette mutagenesis [Wells *et al.*, Gene, 34:315 (1985)], restriction selection mutagenesis [Wells *et al.*, Philos. Trans. R. Soc. London SerA, 317:415 (1986)] or other known techniques can be performed on the cloned DNA to produce the XCRF variant DNA.

Scanning amino acid analysis can also be employed to identify one or more amino acids along a contiguous sequence. Among the preferred scanning amino acids are relatively small, neutral amino acids. Such amino acids include alanine, glycine, serine, and cysteine. Alanine is typically a preferred scanning amino acid among this group because it eliminates the side-chain beyond the beta-carbon and is less likely to alter the main chain conformation of the variant [Cunningham and Wells, Science, 244: 1081-1085 (1989)]. Alanine is also typically preferred because it is the most common amino acid. Further, it is frequently found in both buried and exposed positions [Creighton, The Proteins, (W.H. Freeman & Co., N.Y.); Chothia, J. Mol. Biol., 150:1 (1976)]. If alanine substitution does not yield adequate amounts of variant, an isoteric amino acid can be used.

Amino acids in the XCRF polypeptide sequences of the invention that are essential for function can also be identified by methods known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis (*see, e.g.*, Cunningham, et al. (1989) Science 244:1081-5). The latter procedure introduces single alanine mutations at every residue in the molecule. The resulting mutant molecules are then tested for metabolic-related activity using assays as described above. Of special interest are substitutions of charged amino acids with other charged or neutral amino acids that may produce proteins with highly desirable improved characteristics, such as less aggregation. Aggregation may not only reduce activity but also be problematic when preparing pharmaceutical or physiologically acceptable formulations, because aggregates can be immunogenic (*see, e.g.*, Pinckard, et al., (1967) Clin Exp Immunol 2:331-340; Robbins, et al., (1987) Diabetes 36:838-41; and Cleland, et al., (1993) Crit Rev Ther Drug Carrier Syst 10:307-77).

Thus, the fragment, derivative, analog, or homolog of the XCRF polypeptides of the present invention may be, for example: (i) one in which one or more of the amino acid residues are substituted

with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue) and such substituted amino acid residue may or may not be one encoded by the genetic code (i.e. may be a non-naturally occurring amino acid); or (ii) one in which one or more of the amino acid residues includes a substituent group; or (iii) one in which the XCRF polypeptides are fused with another compound, such as a compound to increase the half-life of the fragment (for example, polyethylene glycol); or (iv) one in which the additional amino acids are fused to the above form of the fragment , such as an IgG Fc fusion region peptide or leader or secretory sequence or a sequence which is employed for purification of the above form of the fragment or a pro-protein sequence. Such fragments, derivatives and analogs are deemed to be within the scope of those skilled in the art from the teachings herein.

A further embodiment of the invention relates to a polypeptide which comprises the amino acid sequence of XCRF polypeptides having an amino acid sequence which contains at least one conservative amino acid substitution, but not more than 50 conservative amino acid substitutions, not more than 40 conservative amino acid substitutions, not more than 30 conservative amino acid substitutions, and not more than 20 conservative amino acid substitutions. Also provided are polypeptides which comprise the amino acid sequence of an XCRF fragment, having at least one, but not more than 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 conservative amino acid substitutions.

In addition, amino acids have chirality within the body of either L or D. In some embodiments it is preferable to alter the chirality of the amino acids in the XCRF polypeptide fragments of the invention in order to extend half-life within the body. Thus, in some embodiments, one or more of the amino acids are preferably in the L configuration. In other embodiments, one or more of the amino acids are preferably in the D configuration.

Percent Identity

The polypeptides of the present invention also include polypeptides having an amino acid sequence at least 50% identical, at least 60% identical, or 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to an XCRF polypeptide as described above. By a polypeptide having an amino acid sequence at least, for example, 95% "identical" to an XCRF polypeptide amino acid sequence is meant that the amino acid sequence is identical to the XCRF polypeptide sequence except that it may include up to five amino acid alterations per each 100 amino acids of the XCRF polypeptide amino acid sequence. The reference sequence is the XCRF polypeptide with a sequence corresponding to the sequence provided in SEQ ID NO: 2, 4, 6 or 8. Thus, to obtain a polypeptide having an amino acid sequence at least 95% identical to an XCRF polypeptide amino acid sequence, up to 5% (5 of 100) of the amino acid residues in the sequence may be inserted, deleted, or substituted with

another amino acid compared with the XCRF polypeptide sequence. These alterations may occur at the amino or carboxy termini or anywhere between those terminal positions, interspersed either individually among residues in the sequence or in one or more contiguous groups within the sequence.

As a practical matter, whether any particular polypeptide is a percentage identical to an XCRF polypeptide can be determined conventionally using known computer programs. Such algorithms and programs include, but are by no means limited to, TBLASTN, BLASTP, FASTA, TFASTA, and CLUSTALW (Pearson and Lipman, (1988) Proc Natl Acad Sci USA 85:2444-8; Altschul et al., (1990) J Mol Biol 215:403-410; Thompson et al., (1994) Nucleic Acids Res 22(2):4673-4680; Higgins et al, (1996) Meth Enzymol 266:383-402; Altschul et al, (1997) Nucleic Acids Res 25:3389-3402; Altschul et al., (1993) Nature Genetics 3:266-272). In a particularly preferred embodiment, protein and nucleic acid sequence homologies are evaluated using the Basic Local Alignment Search Tool ("BLAST"), which is well known in the art (*See, e.g.*, Karlin and Altschul (1990) Proc Natl Acad Sci USA 87:2264-8; Altschul et al., 1990, 1993, 1997, all *supra*). In particular, five specific BLAST programs are used to perform the following tasks:

(1) BLASTP and BLAST3 compare an amino acid query sequence against a protein sequence database;

(2) BLASTN compares a nucleotide query sequence against a nucleotide sequence database;

(3) BLASTX compares the six-frame conceptual translation products of a query nucleotide sequence (both strands) against a protein sequence database;

(4) TBLASTN compares a query protein sequence against a nucleotide sequence database translated in all six reading frames (both strands); and

(5) TBLASTX compares the six-frame translations of a nucleotide query sequence against the six-frame translations of a nucleotide sequence database.

The BLAST programs identify homologous sequences by identifying similar segments, which are referred to herein as "high-scoring segment pairs," between a query amino or nucleic acid sequence and a test sequence which is preferably obtained from a protein or nucleic acid sequence database. High-scoring segment pairs are preferably identified (*i.e.*, aligned) by means of a scoring matrix, many of which are known in the art. Preferably, the scoring matrix used is the BLOSUM62 matrix (see, Gonnet et al., (1992) Science 256:1443-5; Henikoff and Henikoff (1993) Proteins 17:49-61). Less preferably, the PAM or PAM250 matrices may also be used (*See, e.g.*, Schwartz and Dayhoff, eds, (1978) Matrices for Detecting Distance Relationships: Atlas of Protein Sequence and Structure, Washington: National Biomedical Research Foundation). The BLAST programs evaluate the statistical significance of all high-scoring segment pairs identified, and preferably selects those segments which satisfy a user-specified threshold of significance, such as a user-specified percent homology. Preferably,

the statistical significance of a high-scoring segment pair is evaluated using the statistical significance formula of Karlin (*See, e.g.*, Karlin and Altschul, (1990) Proc Natl Acad Sci USA 87:2264-8). The BLAST programs may be used with the default parameters or with modified parameters provided by the user. Preferably, the parameters are default parameters.

A preferred method for determining the best overall match between a query sequence (a sequence of the present invention) and a subject sequence, also referred to as a global sequence alignment, can be determined using the FASTDB computer program based on the algorithm of Brutlag et al. (1990) Comp App Biosci 6:237-245. In a sequence alignment the query and subject sequences are both amino acid sequences. The result of said global sequence alignment is in percent identity. Preferred parameters used in a FASTDB amino acid alignment are: Matrix=PAM 0, k-tuple=2, Mismatch Penalty=1, Joining Penalty=20, Randomization Group=25 Length=0, Cutoff Score=1, Window Size=sequence length, Gap Penalty=5, Gap Size Penalty=0.05, Window Size=247 or the length of the subject amino acid sequence, whichever is shorter.

If the subject sequence is shorter than the query sequence due to N-or C-terminal deletions, not because of internal deletions, the results, in percent identity, must be manually corrected because the FASTDB program does not account for N- and C-terminal truncations of the subject sequence when calculating global percent identity. For subject sequences truncated at the N- and C-termini, relative to the query sequence, the percent identity is corrected by calculating the number of residues of the query sequence that are N- and C- terminal of the subject sequence, that are not matched/aligned with a corresponding subject residue, as a percent of the total bases of the query sequence. Whether a residue is matched/aligned is determined by results of the FASTDB sequence alignment. This percentage is then subtracted from the percent identity, calculated by the above FASTDB program using the specified parameters, to arrive at a final percent identity score. This final percent identity score is what is used for the purposes of the present invention. Only residues to the N- and C-termini of the subject sequence, which are not matched/aligned with the query sequence, are considered for the purposes of manually adjusting the percent identity score. That is, only query amino acid residues outside the farthest N- and C-terminal residues of the subject sequence.

For example, a 90 amino acid residue subject sequence is aligned with a 100-residue query sequence to determine percent identity. The deletion occurs at the N-terminus of the subject sequence and therefore, the FASTDB alignment does not match/align with the first residues at the N-terminus. The 10 unpaired residues represent 10% of the sequence (number of residues at the N- and C- termini not matched/total number of residues in the query sequence) so 10% is subtracted from the percent identity score calculated by the FASTDB program. If the remaining 90 residues were perfectly matched the final percent identity would be 90%.

In another example, a 90-residue subject sequence is compared with a 100-residue query sequence. This time the deletions are internal so there are no residues at the N- or C-termini of the subject sequence, which are not matched/aligned with the query. In this case, the percent identity calculated by FASTDB is not manually corrected. Once again, only residue positions outside the N- and C-terminal ends of the subject sequence, as displayed in the FASTDB alignment, which are not matched/aligned with the query sequence are manually corrected. No other manual corrections are made for the purposes of the present invention.

Production

Note, throughout the disclosure, wherever XCRF polypeptides are discussed, XCRF fragments, variants and derivatives are specifically intended to be included as a preferred subset of XCRF polypeptides.

XCRF polypeptides are preferably isolated from human or mammalian tissue samples or expressed from human or mammalian genes in human or mammalian cells. The XCRF polypeptides of the invention can be made using routine expression methods known in the art. The polynucleotide encoding the desired polypeptide is ligated into an expression vector suitable for any convenient host. Both eukaryotic and prokaryotic host systems are used in forming recombinant polypeptides. The polypeptide is then isolated from lysed cells or from the culture medium and purified to the extent needed for its intended use. Purification is by any technique known in the art, for example, differential extraction, salt fractionation, chromatography, centrifugation, and the like. See, for example, Methods in Enzymology for a variety of methods for purifying proteins.

In a alternative embodiment, the polypeptides of the invention are isolated from milk. The polypeptides can be purified as full-length XCRF polypeptides, which can then be cleaved, if appropriate, *in vitro* to generate an XCRF fragment, or, alternatively, XCRF fragments themselves can be purified from the milk. Any of a large number of methods can be used to purify the present polypeptides from milk, including those taught in Protein Purification Applications, A Practical Approach (New Edition), Edited by Simon Roe, AEA Technology Products and Systems, Biosciences, Harwell; Clark (1998) J Mammary Gland Biol Neoplasia 3:337-50; Wilkins and Velander (1992) 49:333-8; U.S. Patent Nos. 6,140,552; 6,025,540; Hennighausen, Protein Expression and Purification, vol. 1, pp. 3-8 (1990); Harris et al. (1997) Bioseparation 7:31-7; Degener et al. (1998) J Chromatog 799:125-37; Wilkins (1993) J Cell Biochem. Suppl. 0 (17 part A):39; the entire disclosures of each of which are herein incorporated by reference. In a typical embodiment, milk is centrifuged, e.g. at a relatively low speed, to separate the lipid fraction, and the aqueous supernatant is then centrifuged at a higher speed to separate the casein in the milk from the remaining, "whey" fraction. Often, biomedical

proteins are found in this whey fraction, and can be isolated from this fraction using standard chromatographic or other procedures commonly used for protein purification, e.g. as described elsewhere in the present application. In one preferred embodiment, XCRF polypeptides are purified using antibodies specific to XCRF polypeptides, e.g. using affinity chromatography. In addition, methods can be used to isolate particular XCRF fragments, e.g. electrophoretic or other methods for isolating proteins of a particular size. The XCRF polypeptides isolating using these methods can be naturally occurring, as XCRF polypeptides have been discovered to be naturally present in the milk of mammals, or can be the result of the recombinant production of the protein in the mammary glands of a non-human mammal, as described *infra*. In one such embodiment, the XCRF is produced as a fusion protein with a heterologous, antigenic polypeptide sequence, which antigenic sequence can be used to purify the protein, e.g., using standard immuno-affinity methodology.

In addition, shorter protein fragments may be produced by chemical synthesis. Alternatively, the proteins of the invention are extracted from cells or tissues of humans or non-human animals. Methods for purifying proteins are known in the art, and include the use of detergents or chaotropic agents to disrupt particles followed by differential extraction and separation of the polypeptides by ion exchange chromatography, affinity chromatography, sedimentation according to density, and gel electrophoresis.

Any XCRF cDNA including that in SEQ ID NO: 1, 3, 5 or 7 can be used to express XCRF polypeptides. The nucleic acid encoding the XCRF polypeptide to be expressed is operably linked to a promoter in an expression vector using conventional cloning technology. The XCRF cDNA insert in the expression vector may comprise the coding sequence for: the full-length XCRF polypeptide; from 6 amino acids to one amino acid less than the full-length XCRF polypeptide; an XCRF polypeptide fragment; or variants and % similar polypeptides.

The expression vector is any of the mammalian, yeast, insect or bacterial expression systems known in the art, some of which are described herein. Commercially available vectors and expression systems are available from a variety of suppliers including Genetics Institute (Cambridge, MA), Stratagene (La Jolla, California), Promega (Madison, Wisconsin), and Invitrogen (San Diego, California). If desired, to enhance expression and facilitate proper protein folding, the codon context and codon pairing of the sequence can be optimized for the particular expression organism into which the expression vector is introduced, as explained by Hatfield, et al., U.S. Patent No. 5,082,767, the disclosures of which are incorporated by reference herein in their entirety.

If the nucleic acid encoding any one of the XCRF polypeptides lacks a methionine to serve as the initiation site, an initiating methionine can be introduced next to the first codon of the nucleic acid using conventional techniques. Similarly, if the insert from the XCRF polypeptide cDNA lacks a poly A signal,

this sequence can be added to the construct by, for example, splicing out the Poly A signal from pSG5 (Stratagene) using BglI and SalI restriction endonuclease enzymes and incorporating it into the mammalian expression vector pXT1 (Stratagene). pXT1 contains the LTRs and a portion of the gag gene from Moloney Murine Leukemia Virus. The position of the LTRs in the construct allows efficient stable transfection. The vector includes the Herpes Simplex Thymidine Kinase promoter and the selectable neomycin gene.

The nucleic acid encoding XCRF can be obtained by PCR from a vector containing the XCRF nucleotide sequence using oligonucleotide primers complementary to the desired XCRF polynucleotide and containing restriction endonuclease sequences for Pst I incorporated into the 5' primer and BglII at the 5' end of the corresponding cDNA 3' primer, taking care to ensure that the sequence encoding the XCRF is positioned properly with respect to the poly A signal. The purified polynucleotide obtained from the resulting PCR reaction is digested with PstI, blunt ended with an exonuclease, digested with Bgl II, purified and ligated to pXT1, now containing a poly A signal and digested with BglII.

Transfection of an XCRF expressing vector into mouse NIH 3T3 cells is one embodiment of introducing polynucleotides into host cells. Introduction of a polynucleotide encoding a polypeptide into a host cell can be effected by calcium phosphate transfection, DEAE-dextran mediated transfection, cationic lipid-mediated transfection, electroporation, transduction, infection, or other methods. Such methods are described in many standard laboratory manuals, such as Davis et al. ((1986) Methods in Molecular Biology, Elsevier Science Publishing Co., Inc., Amsterdam). It is specifically contemplated that the polypeptides of the present invention may in fact be expressed by a host cell lacking a recombinant vector.

A polypeptide of this invention can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most preferably, high performance liquid chromatography ("HPLC") is employed for purification. Polypeptides of the present invention, and preferably the secreted form, can also be recovered from: products purified from natural sources, including bodily fluids, tissues and cells, whether directly isolated or cultured; products of chemical synthetic procedures; and products produced by recombinant techniques from a prokaryotic or eukaryotic host, including, for example, bacterial, yeast, higher plant, insect, and mammalian cells.

Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated or may be non-glycosylated. Preferably the polypeptides of the invention are non-glycosylated. In addition, polypeptides of the invention may also include an initial modified methionine residue, in some cases as a result of host-mediated processes. Thus, it is well

known in the art that the N-terminal methionine encoded by the translation initiation codon generally is removed with high efficiency from any protein after translation in all eukaryotic cells. While the N-terminal methionine on most proteins also is efficiently removed in most prokaryotes, for some proteins, this prokaryotic removal process is inefficient, depending on the nature of the amino acid to which the N-terminal methionine is covalently linked.

In addition to encompassing host cells containing the vector constructs discussed herein, the invention also encompasses primary, secondary, and immortalized host cells of vertebrate origin, particularly mammalian origin, that have been engineered to delete or replace endogenous genetic material (*e.g.*, coding sequence), and/or to include genetic material (*e.g.*, heterologous polynucleotide sequences) that is operably associated with the polynucleotides of the invention, and which activates, alters, and/or amplifies endogenous polynucleotides. For example, techniques known in the art may be used to operably associate heterologous control regions (*e.g.*, promoter and/or enhancer) and endogenous polynucleotide sequences via homologous recombination, see, *e.g.*, U.S. Patent No. 5,641,670, issued June 24, 1997; International Publication No. WO 96/29411, published September 26, 1996; International Publication No. WO 94/12650, published August 4, 1994; Koller et al., (1989) Proc Natl Acad Sci USA 86:8932-5; Koller et al., (1989) Proc Natl Acad Sci USA 86:8927-31; and Zijlstra et al. (1989) Nature 342:435-8; the disclosures of each of which are incorporated by reference in their entireties).

Modifications

In addition, polypeptides of the invention can be chemically synthesized using techniques known in the art (*See, e.g.*, Creighton, 1983 Proteins. New York, New York: W.H. Freeman and Company; and Hunkapiller et al., (1984) Nature 310:105-11). For example, a relative short fragment of the invention can be synthesized by use of a peptide synthesizer. Furthermore, if desired, nonclassical amino acids or chemical amino acid analogs can be introduced as a substitution or addition into the fragment sequence. Non-classical amino acids include, but are not limited to, to the D-isomers of the common amino acids, 2,4-diaminobutyric acid, a-amino isobutyric acid, 4-aminobutyric acid, Abu, 2-amino butyric acid, g-Abu, e-Ahx, 6-amino hexanoic acid, Aib, 2-amino isobutyric acid, 3-amino propionic acid, ornithine, norleucine, norvaline, hydroxyproline, sarcosine, citrulline, homocitrulline, cysteic acid, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine, b-alanine, fluoroamino acids, designer amino acids such as b-methyl amino acids, Ca-methyl amino acids, Na-methyl amino acids, and amino acid analogs in general. Furthermore, the amino acid can be D (dextrorotary) or L (levorotary).

The invention encompasses polypeptides which are differentially modified during or after translation, *e.g.*, by glycosylation, acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to an antibody molecule or other cellular ligand, etc. Any of numerous chemical modifications may be carried out by known techniques, including but not limited to, specific chemical cleavage by cyanogen bromide, trypsin, chymotrypsin, papain, V8 protease, NaBH4; acetylation, formylation, oxidation, reduction; metabolic synthesis in the presence of tunicamycin; etc.

Additional post-translational modifications encompassed by the invention include, for example, N-linked or O-linked carbohydrate chains, processing of N-terminal or C-terminal ends), attachment of chemical moieties to the amino acid backbone, chemical modifications of N-linked or O-linked carbohydrate chains, and addition or deletion of an N-terminal methionine residue as a result of procaryotic host cell expression. The polypeptides may also be modified with a detectable label, such as an enzymatic, fluorescent, isotopic or affinity label to allow for detection and isolation of the polypeptide.

Also provided by the invention are chemically modified derivatives of the polypeptides of the invention that may provide additional advantages such as increased solubility, stability and circulating time of the polypeptide, or decreased immunogenicity. See U.S. Patent No: 4,179,337. The chemical moieties for derivitization may be selected from water soluble polymers such as polyethylene glycol, ethylene glycol/propylene glycol copolymers, carboxymethylcellulose, dextran, polyvinyl alcohol and the like. The polypeptides may be modified at random positions within the molecule, or at predetermined positions within the molecule and may include one, two, three or more attached chemical moieties.

The polymer may be of any molecular weight, and may be branched or unbranched. For polyethylene glycol, the preferred molecular weight is between about 1 kDa and about 100 kDa (the term "about" indicating that in preparations of polyethylene glycol, some molecules will weigh more, some less, than the stated molecular weight) for ease in handling and manufacturing. Other sizes may be used, depending on the desired therapeutic profile (e.g., the duration of sustained release desired, the effects, if any on biological activity, the ease in handling, the degree or lack of antigenicity and other known effects of the polyethylene glycol to a therapeutic protein or analog).

The polyethylene glycol molecules (or other chemical moieties) should be attached to the polypeptide with consideration of effects on functional or antigenic domains of the polypeptide. There are a number of attachment methods available to those skilled in the art, e.g., EP 0 401 384, herein incorporated by reference (coupling PEG to G-CSF), see also Malik et al. (1992) Exp Hematol (8):1028-35, reporting pegylation of GM-CSF using tresyl chloride). For example, polyethylene glycol may be

covalently bound through amino acid residues via a reactive group, such as, a free amino or carboxyl group. Reactive groups are those to which an activated polyethylene glycol molecule may be bound. The amino acid residues having a free amino group may include lysine residues and the N-terminal amino acid residues; those having a free carboxyl group may include aspartic acid residues, glutamic acid residues and the C-terminal amino acid residue. Sulfhydryl groups may also be used as a reactive group for attaching the polyethylene glycol molecules. Preferred for therapeutic purposes is attachment at an amino group, such as attachment at the N-terminus or lysine group.

One may specifically desire proteins chemically modified at the N-terminus. Using polyethylene glycol as an illustration of the present composition, one may select from a variety of polyethylene glycol molecules (by molecular weight, branching, etc.), the proportion of polyethylene glycol molecules to protein (polypeptide) molecules in the reaction mix, the type of pegylation reaction to be performed, and the method of obtaining the selected N-terminally pegylated protein. The method of obtaining the N-terminally pegylated preparation (i.e., separating this moiety from other monopegylated moieties if necessary) may be by purification of the N-terminally pegylated material from a population of pegylated protein molecules. Selective proteins chemically modified at the N-terminus may be accomplished by reductive alkylation, which exploits differential reactivity of different types of primary amino groups (lysine versus the N-terminal) available for derivatization in a particular protein. Under the appropriate reaction conditions, substantially selective derivatization of the protein at the N-terminus with a carbonyl group containing polymer is achieved.

Multimers

The polypeptides of the invention may be in monomers or multimers (i.e., dimers, trimers, tetramers and higher multimers). Accordingly, the present invention relates to monomers and multimers of the polypeptides of the invention, their preparation, and compositions (preferably, pharmaceutical or physiologically acceptable compositions) containing them. In specific embodiments, the polypeptides of the invention are monomers, dimers, trimers or tetramers. In additional embodiments, the multimers of the invention are at least dimers, at least trimers, or at least tetramers.

Multimers encompassed by the invention may be homomers or heteromers. As used herein, the term homomer, refers to a multimer containing only polypeptides corresponding to the XCRF polypeptides of the invention (including polypeptide fragments, variants, splice variants, and fusion proteins corresponding to these polypeptide fragments as described herein). These homomers may contain polypeptide fragments having identical or different amino acid sequences. In a specific embodiment, a homomer of the invention is a multimer containing only polypeptide fragments having an identical amino acid sequence. In another specific embodiment, a homomer of the invention is a

multimer containing polypeptide fragments having different amino acid sequences. In specific embodiments, the multimer of the invention is a homotrimer (*e.g.,* containing polypeptides having identical and/or different amino acid sequences). In other specific embodiments, the multimer of the invention is a homohexamer (*e.g.,* containing polypeptides having identical and/or different amino acid sequences). In preferred specific embodiments, the multimer of the invention is a homotrimer containing only polypeptide fragments having an identical amino acid sequence. In other preferred specific embodiments, the multimer of the invention is a homohexamer containing only polypeptide fragments having an identical amino acid sequence. In additional embodiments, the homomeric multimer of the invention is at least a homodimer, at least a homotrimer, at least a homotetramer, or at least a homohexamer.

As used herein, the term heteromer refers to a multimer containing one or more heterologous polypeptides (*i.e.,* corresponding to different proteins or polypeptides thereof) in addition to the polypeptides of the invention. In a specific embodiment, the multimer of the invention is a heterotrimer. In other specific embodiment, the multimer of the invention is a heterohexamer. In additional embodiments, the heteromeric multimer of the invention is at least a heterodimer, at least a heterotrimer, at least a heterotetramer, or at least a heterohexamer.

Multimers of the invention may be the result of hydrophobic, hydrophilic, ionic and/or covalent associations and/or may be indirectly linked, by for example, liposome formation. Thus, in one embodiment, multimers of the invention, such as, for example, homodimers or homotrimers, are formed when polypeptides of the invention contact one another in solution. In another embodiment, heteromultimers of the invention, such as, for example, heterotrimers or heterotetramers, are formed when polypeptides of the invention contact antibodies to the polypeptides of the invention (including antibodies to the heterologous polypeptide sequence in a fusion protein of the invention) in solution. In other embodiments, multimers of the invention are formed by covalent associations with and/or between the polypeptides of the invention. Such covalent associations may involve one or more amino acid residues contained in the polypeptide sequence (e.g., that recited in the sequence listing, or contained in the polypeptide encoded by a deposited clone). In one instance, the covalent associations are cross-linking between cysteine residues located within the polypeptide sequences, which interact in the native (*i.e.*, naturally occurring) polypeptide. In another instance, the covalent associations are the consequence of chemical or recombinant manipulation. Alternatively, such covalent associations may involve one or more amino acid residues contained in the heterologous polypeptide sequence in a fusion protein of the invention.

In one example, covalent associations are between the heterologous sequence contained in a fusion protein of the invention (see, e.g., US Patent Number 5,478,925). In a specific example, the

covalent associations are between the heterologous sequence contained in an Fc fusion protein of the invention (as described herein). In another specific example, covalent associations of fusion proteins of the invention are between heterologous polypeptide sequence from another protein that is capable of forming covalently associated multimers, such as for example, oseteoprotegerin (see, e.g., International Publication NO: WO 98/49305, the contents of which are herein incorporated by reference in its entirety). In another embodiment, two or more polypeptides of the invention are joined through peptide linkers. Examples include those peptide linkers described in U.S. Pat. No. 5,073,627 (hereby incorporated by reference). Proteins comprising multiple polypeptides of the invention separated by peptide linkers may be produced using conventional recombinant DNA technology.

Another method for preparing multimer polypeptides of the invention involves use of polypeptides of the invention fused to a leucine zipper or isoleucine zipper polypeptide sequence. Leucine zipper and isoleucine zipper domains are polypeptides that promote multimerization of the proteins in which they are found. Leucine zippers were originally identified in several DNA-binding proteins, and have since been found in a variety of different proteins (Landschulz et al., (1988) Genes Dev 2:786-800). Among the known leucine zippers are naturally occurring peptides and derivatives thereof that dimerize or trimerize. Examples of leucine zipper domains suitable for producing soluble multimeric proteins of the invention are those described in PCT application WO 94/10308, hereby incorporated by reference. Recombinant fusion proteins comprising a polypeptide of the invention fused to a polypeptide sequence that dimerizes or trimerizes in solution are expressed in suitable host cells, and the resulting soluble multimeric fusion protein is recovered from the culture supernatant using techniques known in the art.

Trimeric polypeptides of the invention may offer the advantage of enhanced biological activity. Preferred leucine zipper moieties and isoleucine moieties are those that preferentially form trimers. One example is a leucine zipper derived from lung surfactant protein D (SPD), as described in Hoppe et al. FEBS Letters (1994) 344:191-5 and in U.S. patent application Ser. No. 08/446,922, hereby incorporated by reference. Other peptides derived from naturally occurring trimeric proteins may be employed in preparing trimeric polypeptides of the invention. In another example, proteins of the invention are associated by interactions between Flag® & polypeptide sequence contained in fusion proteins of the invention containing Flag® polypeptide sequence. In a further embodiment, proteins of the invention are associated by interactions between heterologous polypeptide sequence contained in Flag® fusion proteins of the invention and anti Flag® antibody.

The multimers of the invention may be generated using chemical techniques known in the art. For example, polypeptides desired to be contained in the multimers of the invention may be chemically cross-linked using linker molecules and linker molecule length optimization techniques known in the art

(see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). Additionally, multimers of the invention may be generated using techniques known in the art to form one or more inter-molecule cross-links between the cysteine residues located within the sequence of the polypeptides desired to be contained in the multimer (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). Further, polypeptides of the invention may be routinely modified by the addition of cysteine or biotin to the C-terminus or N-terminus of the polypeptide and techniques known in the art may be applied to generate multimers containing one or more of these modified polypeptides (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). Additionally, at least 30 techniques known in the art may be applied to generate liposomes containing the polypeptide components desired to be contained in the multimer of the invention (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety).

Alternatively, multimers of the invention may be generated using genetic engineering techniques known in the art. In one embodiment, polypeptides contained in multimers of the invention are produced recombinantly using fusion protein technology described herein or otherwise known in the art (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). In a specific embodiment, polynucleotides coding for a homodimer of the invention are generated by ligating a polynucleotide sequence encoding a polypeptide of the invention to a sequence encoding a linker polypeptide and then further to a synthetic polynucleotide encoding the translated product of the polypeptide in the reverse orientation from the original C-terminus to the N-terminus (lacking the leader sequence) (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). In another embodiment, recombinant techniques described herein or otherwise known in the art are applied to generate recombinant polypeptides of the invention which contain a transmembrane domain (or hyrophobic or signal peptide) and which can be incorporated by membrane reconstitution techniques into liposomes (*See, e.g.*, US Patent Number 5,478,925, which is herein incorporated by reference in its entirety).

II. XCRF Polynucleotides of the Invention

Preferred polynucleotides are those that encode XCRF polypeptides of the invention. The recombinant polynucleotides encoding XCRF polypeptides can be used in a variety of ways, including, but not limited to, expressing the polypeptides in recombinant cells for use in screening assays for antagonists and agonists of its activity as well as to facilitate its purification for use in a variety of ways including, but not limited to screening assays for agonists and antagonists of its

activity, diagnostic screens, and raising antibodies, as well as treatment and/or prevention of metabolic-related diseases and disorders and/or to reduce body mass.

The invention relates to the polynucleotides encoding XCRF polypeptides and variant polypeptides thereof as described herein. These polynucleotides may be purified, isolated, and/or recombinant. In all cases, the desired XCRF polynucleotides of the invention are those that encode XCRF polypeptides of the invention having metabolic-related activity as described and discussed herein.

Fragments

A polynucleotide fragment is a polynucleotide having a sequence that entirely is the same as part, but not all, of the full-length XCRF polypeptide or a specified XCRF polypeptide nucleotide sequence. Such fragments may be "free-standing", *i.e.* not part of or fused to other polynucleotides, or they may be comprised within another non-XCRF (heterologous) polynucleotide of which they form a part or region. However, several XCRF polynucleotide fragments may be comprised within a single polynucleotide.

The XCRF polynucleotides of the invention comprise from 18 consecutive bases to the full-length polynucleotide sequences encoding the intact XCRF polypeptide, for example the full-length XCRF polypeptide polynucleotide sequences in SEQ ID NO: 1, 3, 5 or 7. In one aspect of this embodiment, the polynucleotide comprises at least 18, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 120, 125, 130, 135, 140, 145, 150, 155, 160, 165, 170, 175, 180, 185, 190, 195, 200, 205, 210, 215, 220, 225, 230, 235, 240, 245, 250, 255, 260, 265, 270, 275, 280, 285, 290, 295, 300, 305, 310, 315, 320, 325, 330, 335, 340, 345, 350, 355, 360, 365, 370, 375, 380, 385, 390, 395, 400, 405, 410, 415, 420, 425, 430, 435, 440, 445, 450, 455, 460, 465, 470, 475, 480, 485, 490, 495, 500, 505, 510, 515, 520, 525, 530, 535, 540, 545, 550, 555, 560, 565, 570, 575, 580, 585, 590, 595, 600, 605, 610, 615, 620, 625, 630, 635, 640, 645, 650, 655, 660, 665, 670, 675, 680, 685, 690, 695, 700, 705, 710, 715, 720, 725, 730, 735, 740, 745, 750, 755, 760, 765, 770, 775, 780, 785, 790, 795, 800, 805, 810, 815, 820, 825, 830, 835, 840, 845, 850, 855, 860, 865, 870, 875, 880, 885, 890, 895, 900, 905, 910, 915, 920, 925, 930, 935, 940, 945, 950, 955, 960, 965, 970, 975, 980, 985, 990, 995, 1000, 1005, 1010, 1015, 1020, 1025, 1030, 1035, 1040, 1045, 1050, 1055, 1060, 1065, 1070, 1075, 1080, 1085, 1090, 1095, 1100, 1105, 1110, 1115, 1120, 1125, 1130, 1135, 1140, 1145, 1150, 1155, 1160, 1165, 1170, 1175, 1180, 1185, 1190, 1195, 1200, 1205, 1210, 1215, 1220, 1225, 1230, 1235, 1240, 1245, 1250, 1255, 1260, 1265, 1270, 1275, 1280, 1285, 1290, 1295 or 1297 consecutive nucleotides of a polynucleotide of the present invention.

In addition to the above preferred nucleic acid sizes, further preferred nucleic acids comprise at least 18 nucleotides, wherein "at least 18" is defined as any integer between 18 and the integer representing the 3' most nucleotide position of the intact XCRF polypeptide cDNA as set forth in SEQ ID NO: 1 or elsewhere herein.

Further included as preferred polynucleotides of the present invention are nucleic acid fragments at least 18 nucleotides in length, as described above, that are further specified in terms of their 5' and 3' position. The 5' and 3' positions are represented by the position numbers set forth in the sequence listing below. For allelic and degenerate and other variants, position 1 is defined as the 5' most nucleotide of the ORF, i.e., the nucleotide "A" of the start codon (ATG) with the remaining nucleotides numbered consecutively. Therefore, every combination of a 5' and 3' nucleotide position that a polynucleotide fragment, at least 18 contiguous nucleotides in length, could occupy on an intact XCRF polypeptide encoding a polynucleotide of the present invention is included in the invention as an individual species. The polynucleotide fragments specified by 5' and 3' positions can be immediately envisaged and are therefore not individually listed solely for the purpose of not unnecessarily lengthening the specification.

It is noted that the above species of polynucleotide fragments of the present invention may alternatively be described by the formula "x to y"; where "x" equals the 5' most nucleotide position and "y" equals the 3' most nucleotide position of the polynucleotide; and further where "x" equals an integer between 1 and the number of nucleotides of the polynucleotide sequence of the present invention minus 18, and where "y" equals an integer between 19 and the number of nucleotides of the polynucleotide sequence of the present invention minus 18 nucleotides; and where "x" is an integer less than "y" by at least 18.

The present invention also provides for the exclusion of any species of polynucleotide fragments of the present invention specified by 5' and 3' positions or polynucleotides specified by size in nucleotides as described above.

The XCRF polynucleotide fragments of the invention comprise from 18 consecutive bases to the full-length polynucleotide sequence encoding the XCRF polypeptide fragments described in Section II of the Preferred Embodiments of the Invention. In one aspect of this embodiment, the polynucleotide comprises at least 18, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 120, 125, 130, 135, 140, 145, 150, 155, 160, 165, 170, 175, 180, 185, 190, 195, 200, 205, 210, 215, 220, 225, 230, 235, 240, 245, 250, 255, 260, 265, 270, 275, 280, 285, 290, 295, 300, 305, 310, 315, 320, 325, 330, 335, 340, 345, 350, 355, 360, 365, 370, 375, 380, 385, 390, 395, 400, 405, 410, 415, 420, 425, 430, 435, 440, 445, 450, 455, 460, 465, 470, 475, 480, 485, 490, 495, 500, 505, 510, 515, 520, 525, 530, 535, 540, 545, 550, 555, 560, 565, 570, 575, 580, 585, 590, 595, 600,

605, 610, 615, 620, 625, 630, 635, 640, 645, 650, 655, 660, 665, 670, 675, 680, 685, 690, 695, 700, 705, 710, 715, 720, 725, 730, 735, 740, 745, 750, 755, 760, 765, 770, 775, 780, 785, 790, 795, 800, 805, 810, 815, 820, 825, 830, 835, 840, 845, 850, 855, 860, 865, 870, 875, 880, 885, 890, 895, 900, 905, 910, 915, 920, 925, 930, 935, 940, 945, 950, 955, 960, 965, 970, 975, 980, 985, 990, 995, 1000, 1005, 1010, 1015, 1020, 1025, 1030, 1035, 1040, 1045, 1050, 1055, 1060, 1065, 1070, 1075, 1080, 1085, 1090, 1095, 1100, 1105, 1110, 1115, 1120, 1125, 1130, 1135, 1140, 1145, 1150, 1155, 1160, 1165, 1170, 1175, 1180, 1185, 1190, 1195, 1200, 1205, 1210, 1215, 1220, 1225, 1230, 1235, 1240, 1245, 1250, 1255, 1260, 1265, 1270, 1275, 1280, 1285, 1290, 1295 or 1297 consecutive nucleotides of a polynucleotide of the present invention.

In addition to the above preferred nucleic acid sizes, further preferred nucleic acids comprise at least 18 nucleotides, wherein "at least 18" is defined as any integer between 18 and the integer corresponding to the 3' most nucleotide position of an XCRF fragment cDNA herein.

Further included as preferred polynucleotides of the present invention are nucleic acid fragments at least 18 nucleotides in length, as described above, that are further specified in terms of their 5' and 3' position. The 5' and 3' positions are represented by the position numbers set forth in the sequence listing below. For allelic and degenerate and other variants, position 1 is defined as the 5' most nucleotide of the open reading frame (ORF), i.e., the nucleotide "A" of the start codon (ATG) with the remaining nucleotides numbered consecutively. Therefore, every combination of a 5' and 3' nucleotide position that a polynucleotide fragment invention, at least 18 contiguous nucleotides in length, could occupy on an XCRF fragment polynucleotide of the present invention is included in the invention as an individual species. The polynucleotide fragments specified by 5' and 3' positions can be immediately envisaged and are therefore not individually listed solely for the purpose of not unnecessarily lengthening the specification.

It is noted that the above species of polynucleotide fragments of the present invention may alternatively be described by the formula "x to y"; where "x" equals the 5' most nucleotide position and "y" equals the 3' most nucleotide position of the polynucleotide; and further where "x" equals an integer between 1 and the number of nucleotides of the XCRF polynucleotide sequences of the present invention minus 18, and where "y" equals an integer between 9 and the number of nucleotides of the XCRF polynucleotide sequences of the present invention; and where "x" is an integer smaller than "y" by at least 18. . Every combination of "x" and "y" positions are included as specific embodiments of the invention. Moreover, the formula "x" to "y" may be modified as '"x1 – x2" to "y1 – y2"', wherein "x1 – x2" and "y1 – y2" represent positional ranges selected from any two nucleotide positions of the sequence listing. Alternative formulas include '"x1 – x2" to "y"' and '"x" to "y1 – y2"'.

These specific embodiments, and other polynucleotide fragment embodiments described herein may be modified as being "at least", "equal to", "equal to or less than", "less than", "at least __ but not greater than ___" or "from ___ to ___". a specified size or specified 5' and/or 3' positions.

The present invention also provides for the exclusion of any species of polynucleotide fragments of the present invention specified by 5' and 3' positions or polynucleotides specified by size in nucleotides as described above.

Variants

In other preferred embodiments, variants of XCRF polynucleotides encoding XCRF polypeptides are envisioned. Variants of polynucleotides, as the term is used herein, are polynucleotides whose sequence differs from a reference polynucleotide. A variant of a polynucleotide may be a naturally occurring variant such as a naturally occurring allelic variant, or it may be a variant that is not known to occur naturally. Such non-naturally occurring variants of the polynucleotide may be made by mutagenesis techniques, including those applied to polynucleotides, cells or organisms. Generally, differences are limited so that the nucleotide sequences of the reference and the variant are closely similar overall and, in many regions, identical.

Polynucleotide variants that comprise a sequence substantially different from those described above but that, due to the degeneracy of the genetic code, still encode XCRF polypeptides of the present invention are also specifically envisioned. It would also be routine for one skilled in the art to generate the degenerate variants described above, for instance, to optimize codon expression for a particular host (*e.g.,* change codons in the human mRNA to those preferred by other mammalian or bacterial host cells).

As stated above, variant polynucleotides may occur naturally, such as a natural allelic variant, or by recombinant methods. By an "allelic variant" is intended one of several alternate forms of a gene occupying a given locus on a chromosome of an organism (See, *e.g.,* B. Lewin, (1990) Genes IV, Oxford University Press, New York). Non-naturally occurring variants may be produced using art-known mutagenesis techniques. Such nucleic acid variants include those produced by nucleotide substitutions, deletions, or additions. The substitutions, deletions, or additions may involve one or more nucleotides. Alterations in the coding regions may produce conservative or non-conservative amino acid substitutions, deletions or additions. Especially preferred among these are silent substitutions, additions and deletions, which do not alter the properties and activities of XCRF polypeptides of the invention. Also preferred in this regard are conservative substitutions.

Nucleotide changes present in a variant polynucleotide are preferably silent, which means that they do not alter the amino acids encoded by the polynucleotide. However, nucleotide changes may also result in amino acid substitutions, additions, deletions, fusions and truncations in the polypeptide encoded by the reference sequence.

In cases where the nucleotide substitutions result in one or more amino acid changes, preferred XCRF polypeptides include those that retain one or more metabolic-related activity as described in Section I of the Preferred Embodiments of the Invention.

By "retain the same activities" is meant that the activity measured using the polypeptide encoded by the variant XCRF polynucleotide in assays is at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100%, and not more than 101%, 102%, 103%, 104%, 105%, 110%, 115%, 120% or 125% of the activity measured using an XCRF polypeptide described in the Examples Section herein.

By the activity being "increased" is meant that the activity measured using the polypeptide encoded by the variant XCRF polynucleotide in assays is at least 125%, 130%, 135%, 140%, 145%, 150%, 155%, 160%, 170%, 180%, 190%, 200%, 225%, 250%, 275%, 300%, 325%, 350%, 375%, 400%, 450%, or 500% of the activity measured using an XCRF polypeptide described in the Examples Section herein.

By the activity being "decreased" is meant that the activity measured using the polypeptide encoded by the variant XCRF polynucleotide in assays is decreased by at least 25%, 30%, 35%, 40%, 45%, 50%, 75%, 80%, 90% or 95% of the activity measured using an XCRF polypeptidedescribed in the Examples Section herein

Percent Identity

The present invention is further directed to nucleic acid molecules having sequences at least 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98% or 99% identical to the polynucleotide sequences of SEQ ID NO: 1, 3, 5, or 7 or fragments thereof that encode a polypeptide having metabolic-related activity as described in Section I of the Preferred Embodiments of the Invention. Of course, due to the degeneracy of the genetic code, one of ordinary skill in the art will immediately recognize that a large number of the nucleic acid molecules at least 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the nucleic acid sequences shown in SEQ ID NO: 1, 3, 5 or 7 or fragments thereof will encode a polypeptide having biological activity. In fact, since degenerate variants of these nucleotide sequences all encode the same polypeptide, this will be clear to the skilled artisan even without performing the above described comparison assay. It will be further recognized in the art that, for such nucleic acid molecules that are not degenerate variants, a reasonable number will also encode a polypeptide having biological activity. This is because the skilled artisan is fully aware of amino acid substitutions that are either less likely or not likely to significantly affect protein function (*e.g.*, replacing one aliphatic amino acid with a second aliphatic amino acid), as further described previously in Section I of the Preferred Embodiments of the Invention.

By a polynucleotide having a nucleotide sequence at least, for example, 95% "identical" to a reference nucleotide sequence of the present invention, it is intended that the nucleotide sequence of the polynucleotide is identical to the reference sequence except that the polynucleotide sequence may include up to five point mutations per each 100 nucleotides of the reference nucleotide sequence encoding the XCRF polypeptide. In other words, to obtain a polynucleotide having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence may be deleted, inserted, or substituted with another nucleotide. The query sequence may be an entire sequence or any fragment specified as described herein.

The methods of determining and defining whether any particular nucleic acid molecule or polypeptide is at least 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98% or 99% identical to a nucleotide sequence of the present invention can be done by using known computer programs. A preferred method for determining the best overall match between a query sequence (a sequence of the present invention) and a subject sequence, also referred to as a global sequence alignment, can be determined using the FASTDB computer program based on the algorithm of Brutlag et al., ((1990) Comput Appl Biosci. Jul;6(3):237-45). In a sequence alignment the query and subject sequences are both DNA sequences. An RNA sequence can be compared by first converting U's to T's. The result of said global sequence alignment is in percent identity. Preferred parameters used in a FASTDB alignment of DNA sequences to calculate percent identity are: Matrix=Unitary, k-tuple=4, Mismatch Penalty=1, Joining Penalty=30, Randomization Group Length=0, Cutoff Score=1, Gap Penalty=5, Gap Size Penalty 0.05, Window Size=500 or the length of the subject nucleotide sequence, whichever is shorter.

If the subject sequence is shorter than the query sequence because of 5' or 3' deletions, not because of internal deletions, a manual correction must be made to the results. This is because the FASTDB program does not account for 5' and 3' truncations of the subject sequence when calculating percent identity. For subject sequences truncated at the 5' or 3' ends, relative to the query sequence, the percent identity is corrected by calculating the number of bases of the query sequence that are 5' and 3' of the subject sequence, which are not matched/aligned, as a percent of the total bases of the query sequence. Whether a nucleotide is matched/aligned is determined by results of the FASTDB sequence alignment. This percentage is then subtracted from the percent identity, calculated by the above FASTDB program using the specified parameters, to arrive at a final percent identity score. This corrected score is what is used for the purposes of the present invention. Only nucleotides outside the 5' and 3' nucleotides of the subject sequence, as displayed by the FASTDB alignment, which are not matched/aligned with the query sequence, are calculated for the purposes of manually adjusting the percent identity score.

For example, a 90-nucleotide subject sequence is aligned to a 100-nucleotide query sequence to determine percent identity. The deletions occur at the 5' end of the subject sequence and therefore, the FASTDB alignment does not show a matched/alignment of the first 10 nucleotides at 5' end. The 10 unpaired nucleotides represent 10% of the sequence (number of nucleotides at the 5' and 3' ends not matched/total number of nucleotides in the query sequence) so 10% is subtracted from the percent identity score calculated by the FASTDB program. If the remaining 90 nucleotides were perfectly matched the final percent identity would be 90%.

In another example, a 90 nucleotide subject sequence is compared with a 100 nucleotide query sequence. This time the deletions are internal deletions so that there are no nucleotides on the 5' or 3' of the subject sequence which are not matched/aligned with the query. In this case the percent identity calculated by FASTDB is not manually corrected. Once again, only nucleotides 5' and 3' of the subject sequence which are not matched/aligned with the query sequence are manually corrected for. No other manual corrections are made for the purposes of the present invention.

Fusions

Further included in the present invention are polynucleotides encoding the polypeptides of the present invention that are fused in frame to the coding sequences for additional heterologous amino acid sequences. Also included in the present invention are nucleic acids encoding polypeptides of the present invention together with additional, non-coding sequences, including for example, but not limited to non-coding 5' and 3' sequences, vector sequence, sequences used for purification, probing, or priming. For example, heterologous sequences include transcribed, nontranslated sequences that may play a role in transcription, and mRNA processing, for example, ribosome binding and stability of mRNA. The heterologous sequences may alternatively comprise additional coding sequences that provide additional functionalities. Thus, a nucleotide sequence encoding a polypeptide may be fused to a tag sequence, such as a sequence encoding a peptide that facilitates purification of the fused polypeptide. In certain preferred embodiments of this aspect of the invention, the tag amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (QIAGEN, Chatsworth, CA), among others, many of which are commercially available. For instance, hexa-histidine provides for convenient purification of the fusion protein (See, Gentz et al., (1989) Proc Natl Acad Sci USA 86:821-4). The "HA" tag is another peptide useful for purification which corresponds to an epitope derived from the influenza hemagglutinin protein (See, Wilson et al., (1984) Cell 37:767-78). As discussed above, other such fusion proteins include XCRF cDNA fused to Fc at the N- or C-terminus.

III. Recombinant Vectors of the Invention

The term "vector" is used herein to designate either a circular or a linear DNA or RNA molecule, that is either double-stranded or single-stranded, and that comprises at least one polynucleotide of interest that is sought to be transferred in a cell host or in a unicellular or multicellular host organism.

The present invention relates to recombinant vectors comprising any one of the polynucleotides described herein.

The present invention encompasses a family of recombinant vectors that comprise polynucleotides encoding XCRF polypeptides of the invention.

In a first preferred embodiment, a recombinant vector of the invention is used to amplify the inserted polynucleotide in a suitable cell host, this polynucleotide being amplified every time that the recombinant vector replicates. The inserted polynucleotide can be one that encodes XCRF polypeptides of the invention.

A second preferred embodiment of the recombinant vectors according to the invention consists of expression vectors comprising polynucleotides encoding XCRF polypeptides of the invention. Within certain embodiments, expression vectors are employed to express an XCRF polypeptide of the invention, preferably a modified XCRF described in the present invention, which can be then purified and, for example, be used as a treatment for metabolic-related diseases, or simply to reduce body mass of individuals.

Expression requires that appropriate signals are provided in the vectors, said signals including various regulatory elements, such as enhancers/promoters from both viral and mammalian sources, that drive expression of the genes of interest in host cells. Dominant drug selection markers for establishing permanent, stable, cell clones expressing the products are generally included in the expression vectors of the invention, as they are elements that link expression of the drug selection markers to expression of the polypeptide.

More particularly, the present invention relates to expression vectors which include nucleic acids encoding an XCRF polypeptide of the invention, or a modified XCRF as described herein, or variants or fragments thereof, under the control of a regulatory sequence selected among XCRF polypeptides, or alternatively under the control of an exogenous regulatory sequence.

Consequently, preferred expression vectors of the invention are selected from the group consisting of: (a) an XCRF regulatory sequence and driving the expression of a coding polynucleotide operably linked thereto; and (b) an XCRF coding sequence of the invention, operably linked to regulatory sequences allowing its expression in a suitable cell host and/or host organism.

Some of the elements that can be found in the vectors of the present invention are described in further detail in the following sections.

1) General features of the expression vectors of the invention

A recombinant vector according to the invention comprises, but is not limited to, a YAC (Yeast Artificial Chromosome), a BAC (Bacterial Artificial Chromosome), a phage, a phagemid, a cosmid, a plasmid, or even a linear DNA molecule which may consist of a chromosomal, non-chromosomal, semi-synthetic or synthetic DNA. Such a recombinant vector can comprise a transcriptional unit comprising an assembly of :

(1) a genetic element or elements having a regulatory role in gene expression, for example promoters or enhancers. Enhancers are cis-acting elements of DNA, usually from about 10 to 300 bp in length that act on the promoter to increase the transcription;

(2) a structural or coding sequence which is transcribed into mRNA and eventually translated into a polypeptide, said structural or coding sequence being operably linked to the regulatory elements described in (1); and

(3) appropriate transcription initiation and termination sequences. Structural units intended for use in yeast or eukaryotic expression systems preferably include a leader sequence enabling extracellular secretion of translated protein by a host cell. Alternatively, when a recombinant protein is expressed without a leader or transport sequence, it may include a N-terminal residue. This residue may or may not be subsequently cleaved from the expressed recombinant protein to provide a final product.

Generally, recombinant expression vectors will include origins of replication, selectable markers permitting transformation of the host cell, and a promoter derived from a highly expressed gene to direct transcription of a downstream structural sequence. The heterologous structural sequence is assembled in appropriate phase with translation initiation and termination sequences, and preferably a leader sequence capable of directing secretion of the translated protein into the periplasmic space or the extracellular medium. In a specific embodiment wherein the vector is adapted for transfecting and expressing desired sequences in mammalian host cells, preferred vectors will comprise an origin of replication in the desired host, a suitable promoter and enhancer, and also any necessary ribosome binding sites, polyadenylation sites, splice donor and acceptor sites, transcriptional termination sequences, and 5'-flanking non-transcribed sequences. DNA sequences derived from the SV40 viral genome, for example SV40 origin, early promoter, enhancer, splice and polyadenylation sites may be used to provide the required non-transcribed genetic elements.

2) Regulatory elements

Promoters

The suitable promoter regions used in the expression vectors of the present invention are chosen taking into account the cell host in which the heterologous gene is expressed. The particular promoter employed to control the expression of a nucleic acid sequence of interest is not believed to be important, so long as it is capable of directing the expression of the nucleic acid in the targeted cell. Thus, where a human cell is targeted, it is preferable to position the nucleic acid coding region adjacent to and under the control of a promoter that is capable of being expressed in a human cell, such as, for example, a human or a viral promoter.

A suitable promoter may be heterologous with respect to the nucleic acid for which it controls the expression or alternatively can be endogenous to the native polynucleotide containing the coding sequence to be expressed. Additionally, the promoter is generally heterologous with respect to the recombinant vector sequences within which the construct promoter/coding sequence has been inserted.

Promoter regions can be selected from any desired gene using, for example, CAT (chloramphenicol transferase) vectors and more preferably pKK232-8 and pCM7 vectors. Preferred bacterial promoters are the LacI, LacZ, the T3 or T7 bacteriophage RNA polymerase promoters, the gpt, lambda PR, PL and trp promoters (EP 0036776), the polyhedrin promoter, or the p10 protein promoter from baculovirus (Kit Novagen) (Smith et al., (1983) Mol Cell Biol 3:2156-65; O'Reilly et al., 1992), the lambda PR promoter or also the trc promoter.

Eukaryotic promoters include CMV immediate early, HSV thymidine kinase, early and late SV40, LTRs from retrovirus, and mouse metallothionein-L. In addition, promoters specific for a particular cell type may be chosen, such as those facilitating expression in adipose tissue, muscle tissue, or liver. Selection of a convenient vector and promoter is well within the level of ordinary skill in the art.

The choice of a promoter is well within the ability of a person skilled in the field of genetic engineering. For example, one may refer to Sambrook et al. (1989) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, NY, Vol. 1, 2, 3 (1989), or also to the procedures described by Fuller et al. (1996) Immunology in Current Protocols in Molecular Biology.

Other regulatory elements

Where a cDNA insert is employed, one will typically desire to include a polyadenylation signal to effect proper polyadenylation of the gene transcript. The nature of the polyadenylation signal is not believed to be crucial to the successful practice of the invention, and any such sequence may be employed such as human growth hormone and SV40 polyadenylation signals. Also contemplated as an

element of the expression cassette is a terminator. These elements can serve to enhance message levels and to minimize read through from the cassette into other sequences.

Vectors containing the appropriate DNA sequence as described above can be utilized to transform an appropriate host to allow the expression of the desired polypeptide or polynucleotide.

3) Selectable markers

Such markers would confer an identifiable change to the cell permitting easy identification of cells containing the expression construct. The selectable marker genes for selection of transformed host cells are preferably dihydrofolate reductase or neomycin resistance for eukaryotic cell culture, TRP1 for S. cerevisiae or tetracycline, rifampicin or ampicillin resistance in *E. coli*, or levan saccharase for mycobacteria, this latter marker being a negative selection marker.

4) Preferred vectors

Bacterial vectors

As a representative but non-limiting example, useful expression vectors for bacterial use can comprise a selectable marker and a bacterial origin of replication derived from commercially available plasmids comprising genetic elements of pBR322 (ATCC 37017). Such commercial vectors include, for example, pKK223-3 (Pharmacia, Uppsala, Sweden), and pGEM1 (Promega Biotec, Madison, WI, USA).

Large numbers of other suitable vectors are known to those of skill in the art, and are commercially available, such as the following bacterial vectors: pQE70, pQE60, pQE-9 (Qiagen), pbs, pD10, phagescript, psiX174, pbluescript SK, pbsks, pNH8A, pNH16A, pNH18A, pNH46A (Stratagene); ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5 (Pharmacia); pWLNEO, pSV2CAT, pOG44, pXT1, pSG (Stratagene); pSVK3, pBPV, pMSG, pSVL (Pharmacia); pQE-30 (QIAexpress).

Baculovirus vectors

A suitable vector for the expression of polypeptides of the invention is a baculovirus vector that can be propagated in insect cells and in insect cell lines. A specific suitable host vector system is the pVL1392/1393 baculovirus transfer vector (Pharmingen) that is used to transfect the SF9 cell line (ATCC N°CRL 1711) which is derived from Spodoptera frugiperda.

Other suitable vectors for the expression of an XCRF polypeptide comprising the globular C-terminal C1q homology domain in a baculovirus expression system include those described by Chai et al. (1993; Biotechnol Appl Biochem 18 (Pt 3):259-73); Vlasak et al. (1983; Eur J Biochem 135:123-6); and Lenhard et al. (1996; Gene 169:187-90).

Plasmid vectors

A suitable vector for the expression of polypeptides of the invention is a plasmid vector that contains an SV40-derived origin of replication and that can be used for transient transfection of COS cells (ATCC N°CRL1650; N°CRL1651). Plasmid vectors suitable for transient transfection of COS cells include but are not limited to CDM8 (Invitrogen).

Viral vectors

In one specific embodiment, the vector is derived from an adenovirus. Preferred adenovirus vectors according to the invention are those described by Feldman and Steg (1996; Semin Interv Cardiol 1:203-8) or Ohno et al. (1994; Science 265:781-4). Another preferred recombinant adenovirus according to this specific embodiment of the present invention is the human adenovirus type 2 or 5 (Ad 2 or Ad 5) or an adenovirus of animal origin (French patent application No. FR-93.05954).

Retrovirus vectors and adeno-associated virus vectors are generally understood to be the recombinant gene delivery systems of choice for the transfer of exogenous polynucleotides *in vivo*, particularly to mammals, including humans. These vectors provide efficient delivery of genes into cells, and the transferred nucleic acids are stably integrated into the chromosomal DNA of the host.

Particularly preferred retroviruses for the preparation or construction of retroviral *in vitro* or *in vivo* gene delivery vehicles of the present invention include retroviruses selected from the group consisting of Mink-Cell Focus Inducing Virus, Murine Sarcoma Virus, Reticuloendotheliosis virus and Rous Sarcoma virus. Particularly preferred Murine Leukemia Viruses include the 4070A and the 1504A viruses, Abelson (ATCC No VR-999), Friend (ATCC No VR-245), Gross (ATCC No VR-590), Rauscher (ATCC No VR-998) and Moloney Murine Leukemia Virus (ATCC No VR-190; PCT Application No WO 94/24298). Particularly preferred Rous Sarcoma Viruses include Bryan high titer (ATCC Nos VR-334, VR-657, VR-726, VR-659 and VR-728). Other preferred retroviral vectors are those described in Roth et al. (1996), PCT Application No WO 93/25234, PCT Application No WO 94/ 06920, Roux et al., ((1989) Proc Natl Acad Sci U S A 86:9079-83), Julan et al., (1992) J Gen. Virol 3:3251-3255 and Neda et al., ((1991) J Biol Chem 266:14143-6).

Yet another viral vector system that is contemplated by the invention consists of the adeno-associated virus (AAV). The adeno-associated virus is a naturally occurring defective virus that requires another virus, such as an adenovirus or a herpes virus, as a helper virus for efficient replication and a productive life cycle (Muzyczka et al., (1992) Curr Top Microbiol Immunol 158:97-129). It is also one of the few viruses that may integrate its DNA into non-dividing cells, and exhibits a high frequency of stable integration (Flotte et al., (1992) Am J Respir Cell Mol Biol 7:349-56; Samulski et

al., (1989) J Virol 63:3822-8; McLaughlin et al., (1989) Am J Hum Genet 59:561-569). One advantageous feature of AAV derives from its reduced efficacy for transducing primary cells relative to transformed cells.

5) Delivery of the recombinant vectors

In order to effect expression of the polynucleotides of the invention, these constructs must be delivered into a cell. This delivery may be accomplished *in vitro*, as in laboratory procedures for transforming cell lines, or *in vivo* or *ex vivo*, as in the treatment of certain disease states.

One mechanism is viral infection where the expression construct is encapsulated in an infectious viral particle.

Several non-viral methods for the transfer of polynucleotides into cultured mammalian cells are also contemplated by the present invention, and include, without being limited to, calcium phosphate precipitation (Graham et al., (1973) Virology 54:536-9; Chen et al., (1987) Mol Cell Biol 7:2745-52), DEAE-dextran (Gopal, (1985) Mol Cell Biol 5:1188-90), electroporation (Tur-Kaspa et al., (1986) Mol Cell Biol 6:716-8; Potter et al., (1984) Proc Natl Acad Sci USA 81:7161-5.), direct microinjection (Harland et al., (1985) J Cell Biol 101:1094-9), DNA-loaded liposomes (Nicolau et al., (1982) Biochim Biophys Acta 721:185-90; Fraley et al., (1979) Proc Natl Acad Sci USA 76:3348-52), and receptor-mediated transfection (Wu and Wu, (1987) J Biol Chem 262:4429-32; Wu and Wu (1988) Biochemistry 27:887-92). Some of these techniques may be successfully adapted for *in vivo* or *ex vivo* use.

Once the expression polynucleotide has been delivered into the cell, it may be stably integrated into the genome of the recipient cell. This integration may be in the cognate location and orientation via homologous recombination (gene replacement) or it may be integrated in a random, non-specific location (gene augmentation). In yet further embodiments, the nucleic acid may be stably maintained in the cell as a separate, episomal segment of DNA. Such nucleic acid segments or "episomes" encode sequences sufficient to permit maintenance and replication independent of or in synchronization with the host cell cycle.

One specific embodiment for a method for delivering a protein or peptide to the interior of a cell of a vertebrate *in vivo* comprises the step of introducing a preparation comprising a physiologically acceptable carrier and a naked polynucleotide operatively coding for the polypeptide of interest into the interstitial space of a tissue comprising the cell, whereby the naked polynucleotide is taken up into the interior of the cell and has a physiological effect. This is particularly applicable for transfer *in vitro* but it may be applied to *in vivo* as well.

Compositions for use *in vitro* and *in vivo* comprising a "naked" polynucleotide are described in PCT application No. WO 90/11092 (Vical Inc.) and also in PCT application No. WO 95/11307 (Institut

Pasteur, INSERM, Université d'Ottawa) as well as in the articles of Tascon et al. (1996) Nature Medicine 2:888-892 and of Huygen et al. ((1996) Nat Med 2:893-8).

In still another embodiment of the invention, the transfer of a naked polynucleotide of the invention, including a polynucleotide construct of the invention, into cells may be proceeded with a particle bombardment (biolistic), said particles being DNA-coated microprojectiles accelerated to a high velocity allowing them to pierce cell membranes and enter cells without killing them, such as described by Klein et al. ((1990) Curr Genet 17:97-103).

In a further embodiment, the polynucleotide of the invention may be entrapped in a liposome (Ghosh and Bacchawat, (1991) Targeted Diagn Ther 4:87-103; Wong et al., (1980) Gene 10:87-94; Nicolau et al., (1987) Methods Enzymol.149:157-76). These liposomes may further be targeted to cells expressing LSR by incorporating leptin, triglycerides, or other known LSR ligands into the liposome membrane.

In a specific embodiment, the invention provides a composition for the *in vivo* production of an XCRF globular head polypeptide described herein. It comprises a naked polynucleotide operatively coding for this polypeptide, in solution in a physiologically acceptable carrier, and suitable for introduction into a tissue to cause cells of the tissue to express the said polypeptide.

The amount of vector to be injected to the desired host organism varies according to the site of injection. As an indicative dose, it will be injected between 0.1 and 100 μg of the vector in an animal body, preferably a mammal body, for example a mouse body.

In another embodiment of the vector according to the invention, it may be introduced *in vitro* in a host cell, preferably in a host cell previously harvested from the animal to be treated and more preferably a somatic cell such as a muscle cell. In a subsequent step, the cell that has been transformed with the vector coding for the desired XCRF globular head polypeptide or the desired fragment thereof is reintroduced into the animal body in order to deliver the recombinant protein within the body either locally or systemically.

IV. Recombinant Cells of the Invention

Another object of the invention consists of host cells recombinant for, i.e., that have been transformed or transfected with one of the polynucleotides described herein, and more precisely a polynucleotide comprising a polynucleotide encoding an XCRF polypeptide of the invention such as any one of those described in "Polynucleotides of the Invention". These polynucleotides can be present in cells as a result of transient or stable transfection. The invention includes host cells that are transformed (prokaryotic cells) or that are transfected (eukaryotic cells) with a recombinant vector such as any one of those described in "Recombinant Vectors of the Invention".

Generally, a recombinant host cell of the invention comprises at least one of the polynucleotides or the recombinant vectors of the invention that are described herein.

Preferred host cells used as recipients for the recombinant vectors of the invention are the following :

a) Prokaryotic host cells: *Escherichia coli* strains (I.E. DH5-α strain), *Bacillus subtilis*, *Salmonella typhimurium*, and strains from species like *Pseudomonas*, *Streptomyces* and *Staphylococcus*, and

b) Eukaryotic host cells: HeLa cells (ATCC N°CCL2; N°CCL2.1; N°CCL2.2), Cv 1 cells (ATCC N°CCL70), COS cells (ATCC N°CRL1650; N°CRL1651), Sf-9 cells (ATCC N°CRL1711), C127 cells (ATCC N° CRL-1804), 3T3 (ATCC N° CRL-6361), CHO (ATCC N° CCL-61), human kidney 293 (ATCC N° 45504; N° CRL-1573), BHK (ECACC N° 84100501; N° 84111301), PLC cells, HepG2, and Hep3B.

The constructs in the host cells can be used in a conventional manner to produce the gene product encoded by the recombinant sequence.

Following transformation of a suitable host and growth of the host to an appropriate cell density, the selected promoter is induced by appropriate means, such as temperature shift or chemical induction, and cells are cultivated for an additional period.

In a preferred embodiment, recombinant protein expressed by cells that have been stably or transiently transfected with a recombinant vector such as any one of those described in "Recombinant Vectors of the Invention" is recovered from culture supernatant.

Alternatively, cells may be harvested (typically by centrifugation), disrupted by physical or chemical means, and the resulting crude extract retained for further purification.

Microbial cells employed in the expression of proteins can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents. Such methods are well known by the skilled artisan.

Further, according to the invention, these recombinant cells can be created *in vitro* or *in vivo* in an animal, preferably a mammal, most preferably selected from the group consisting of mice, rats, dogs, pigs, sheep, cattle, and primates, not to include humans. Recombinant cells created *in vitro* can also be later surgically implanted in an animal, for example. Methods to create recombinant cells *in vivo* in animals are well-known in the art.

The present invention also encompasses primary, secondary, and immortalized homologously recombinant host cells of vertebrate origin, preferably mammalian origin and particularly human origin, that have been engineered to: a) insert exogenous (heterologous) polynucleotides into the endogenous chromosomal DNA of a targeted gene, b) delete endogenous chromosomal DNA, and/or c) replace

endogenous chromosomal DNA with exogenous polynucleotides. Insertions, deletions, and/or replacements of polynucleotide sequences may be to the coding sequences of the targeted gene and/or to regulatory regions, such as promoter and enhancer sequences, operably associated with the targeted gene.

The present invention further relates to a method of making a homologously recombinant host cell *in vitro* or *in vivo*, wherein the expression of a targeted gene not normally expressed in the cell is altered. Preferably the alteration causes expression of the targeted gene under normal growth conditions or under conditions suitable for producing the polypeptide encoded by the targeted gene. The method comprises the steps of: (a) transfecting the cell *in vitro* or *in vivo* with a polynucleotide construct, the polynucleotide construct comprising; (i) a targeting sequence; (ii) a regulatory sequence and/or a coding sequence; and (iii) an unpaired splice donor site, if necessary, thereby producing a transfected cell; and (b) maintaining the transfected cell *in vitro* or *in vivo* under conditions appropriate for homologous recombination.

The present invention further relates to a method of altering the expression of a targeted gene in a cell *in vitro* or *in vivo* wherein the gene is not normally expressed in the cell, comprising the steps of: (a) transfecting the cell *in vitro* or *in vivo* with a polynucleotide construct, the polynucleotide construct comprising: (i) a targeting sequence; (ii) a regulatory sequence and/or a coding sequence; and (iii) an unpaired splice donor site, if necessary, thereby producing a transfected cell; and (b) maintaining the transfected cell *in vitro* or *in vivo* under conditions appropriate for homologous recombination, thereby producing a homologously recombinant cell; and (c) maintaining the homologously recombinant cell *in vitro* or *in vivo* under conditions appropriate for expression of the gene.

The present invention further relates to a method of making a polypeptide of the present invention by altering the expression of a targeted endogenous gene in a cell *in vitro* or *in vivo* wherein the gene is not normally expressed in the cell, comprising the steps of: a) transfecting the cell *in vitro* with a polynucleotide construct, the polynucleotide construct comprising: (i) a targeting sequence; (ii) a regulatory sequence and/or a coding sequence; and (iii) an unpaired splice donor site, if necessary, thereby producing a transfected cell; (b) maintaining the transfected cell *in vitro* or *in vivo* under conditions appropriate for homologous recombination, thereby producing a homologously recombinant cell; and c) maintaining the homologously recombinant cell *in vitro* or *in vivo* under conditions appropriate for expression of the gene thereby making the polypeptide.

The present invention further relates to a polynucleotide construct that alters the expression of a targeted gene in a cell type in which the gene is not normally expressed. This occurs when a polynucleotide construct is inserted into the chromosomal DNA of the target cell, wherein the polynucleotide construct comprises: a) a targeting sequence; b) a regulatory sequence and/or coding

sequence; and c) an unpaired splice-donor site, if necessary. Further included are polynucleotide constructs, as described above, wherein the construct further comprises a polynucleotide that encodes a polypeptide and is in-frame with the targeted endogenous gene after homologous recombination with chromosomal DNA.

The compositions may be produced, and methods performed, by techniques known in the art, such as those described in U.S. Patent Nos: 6,054,288; 6,048,729; 6,048,724; 6,048,524; 5,994,127; 5,968,502; 5,965,125; 5,869,239; 5,817,789; 5,783,385; 5,733,761; 5,641,670; 5,580,734 ; International Publication Nos:WO96/29411, WO 94/12650; and scientific articles described by Koller et al., (1994) Annu Rev Immunol 10:705-730; the disclosures of each of which are incorporated by reference in their entireties).

The expression of XCRF in mammalian, and typically human, cells may be rendered defective, or alternatively it may be enhanced, with the insertion of an XCRF genomic or cDNA sequence with the replacement of the XCRF gene counterpart in the genome of an animal cell by an XCRF polynucleotide according to the invention. These genetic alterations may be generated by homologous recombination events using specific DNA constructs that have been previously described.

One kind of host cell that may be used are mammalian zygotes, such as murine zygotes. For example, murine zygotes may undergo microinjection with a purified DNA molecule of interest, for example a purified DNA molecule that has previously been adjusted to a concentration range from 1 ng/ml –for BAC inserts- 3 ng/μl –for P1 bacteriophage inserts- in 10 mM Tris-HCl, pH 7.4, 250 μM EDTA containing 100 mM NaCl, 30 μM spermine, and 70 μM spermidine. When the DNA to be microinjected has a large size, polyamines and high salt concentrations can be used in order to avoid mechanical breakage of this DNA, as described by Schedl et al ((1993) Nature 362:258-61).

Any one of the polynucleotides of the invention, including the DNA constructs described herein, may be introduced in an embryonic stem (ES) cell line, preferably a mouse ES cell line. ES cell lines are derived from pluripotent, uncommitted cells of the inner cell mass of pre-implantation blastocysts. Preferred ES cell lines are the following: ES-E14TG2a (ATCC No.CRL-1821), ES-D3 (ATCC No.CRL1934 and No. CRL-11632), YS001 (ATCC No. CRL-11776), 36.5 (ATCC No. CRL-11116). To maintain ES cells in an uncommitted state, they are cultured in the presence of growth inhibited feeder cells which provide the appropriate signals to preserve this embryonic phenotype and serve as a matrix for ES cell adherence. Preferred feeder cells are primary embryonic fibroblasts that are established from tissue of day 13- day 14 embryos of virtually any mouse strain, that are maintained in culture, such as described by Abbondanzo et al. (1993; Methods Enzymol 225:803-23) and are inhibited in growth by irradiation, such as described by Robertson ((1987) Embryo-derived stem cell lines. In: E.J. Robertson Ed. Teratocarcinomas and embrionic stem cells: a practical approach. IRL Press,

Oxford), or by the presence of an inhibitory concentration of LIF, such as described by Pease and Williams (1990; Exp Cell Res 190:209-11).

The constructs in the host cells can be used in a conventional manner to produce the gene product encoded by the recombinant sequence.

Following transformation of a suitable host and growth of the host to an appropriate cell density, the selected promoter is induced by appropriate means, such as temperature shift or chemical induction, and cells are cultivated for an additional period. Cells are typically harvested by centrifugation, disrupted by physical or chemical means, and the resulting crude extract retained for further purification. Microbial cells employed in the expression of proteins can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents. Such methods are well known by the skilled artisan.

V. DNA Construct That Enables Directing Temporal and Spatial XCRF Gene Expression In Transgenic Animals

DNA Constructs Allowing Homologous Recombination: Replacement Vectors

A preferred DNA construct will comprise, from 5'-end to 3'-end: (a) a first nucleotide sequence that is comprised in the XCRF genomic sequence; (b) a nucleotide sequence comprising a positive selection marker, such as the marker for neomycine resistance (*neo*); and (c) a second nucleotide sequence that is comprised in the XCRF genomic sequence, and is located on the genome downstream the first XCRF nucleotide sequence (a).

In a preferred embodiment, this DNA construct also comprises a negative selection marker located upstream the nucleotide sequence (a) or downstream the nucleotide sequence (c). Preferably, the negative selection marker comprises the thymidine kinase (*tk*) gene (Thomas *et al.*, 1986), the hygromycine beta gene (Te Riele *et al.*, 1990), the *hprt* gene (Van der Lugt *et al.*, 1991; Reid *et al.*, 1990) or the Diphteria toxin A fragment (*Dt-A*) gene (Nada *et al.*, 1993; Yagi *et al.* 1990), which disclosures are hereby incorporated by reference in their entireties. Preferably, the positive selection marker is located within an XCRF exon sequence so as to interrupt the sequence encoding an XCRF protein. These replacement vectors are described, for example, by Thomas *et al.* (1986; 1987), Mansour *et al.* (1988) and Koller *et al.* (1992).

The first and second nucleotide sequences (a) and (c) may be indifferently located within an XCRF regulatory sequence, an intronic sequence, an exon sequence or a sequence containing both regulatory and/or intronic and/or exon sequences. The size of the nucleotide sequences (a) and (c) ranges from 1 to 50 kb, preferably from 1 to 10 kb, more preferably from 2 to 6 kb and most preferably

from 2 to 4 kb. Preferably the nucleotide sequences (a) and (c) comprise all or part of SEQ ID NO: 1, 3, 5 or 7.

DNA Constructs Allowing Homologous Recombination: Cre-LoxP System

These new DNA constructs make use of the site specific recombination system of the P1 phage. The P1 phage possesses a recombinase called Cre that interacts specifically with a 34 base pairs *lox*P site. The *lox*P site is composed of two palindromic sequences of 13 bp separated by a 8 bp conserved sequence (Hoess *et al.*, 1986), which disclosure is hereby incorporated by reference in its entirety. The recombination by the Cre enzyme between two *lox*P sites having an identical orientation leads to the deletion of the DNA fragment.

The Cre-*lox*P system used in combination with a homologous recombination technique has been first described by Gu *et al.* (1993, 1994), which disclosures are hereby incorporated by reference in their entireties. Briefly, a nucleotide sequence of interest to be inserted in a targeted location of the genome harbors at least two *lox*P sites in the same orientation and located at the respective ends of a nucleotide sequence to be excised from the recombinant genome. The excision event requires the presence of the recombinase (Cre) enzyme within the nucleus of the recombinant cell host. The recombinase enzyme may be brought at the desired time either by (a) incubating the recombinant cell hosts in a culture medium containing this enzyme, by injecting the Cre enzyme directly into the desired cell, such as described by Araki *et al.* (1995), which disclosure is hereby incorporated by reference in its entirety, or by lipofection of the enzyme into the cells, such as described by Baubonis *et al.* (1993), which disclosure is hereby incorporated by reference in its entirety; (b) transfecting the cell host with a vector comprising the *Cre* coding sequence operably linked to a promoter functional in the recombinant cell host, which promoter being optionally inducible, said vector being introduced in the recombinant cell host, such as described by Gu *et al.* (1993) and Sauer *et al.* (1988), which disclosures are hereby incorporated by reference in their entireties; (c) introducing in the genome of the cell host a polynucleotide comprising the *Cre* coding sequence operably linked to a promoter functional in the recombinant cell host, which promoter is optionally inducible, and said polynucleotide being inserted in the genome of the cell host either by a random insertion event or an homologous recombination event, such as described by Gu *et al.* (1994).

In a specific embodiment, the vector containing the sequence to be inserted in the XCRF gene by homologous recombination is constructed in such a way that selectable markers are flanked by *lox*P sites of the same orientation, it is possible, by treatment by the Cre enzyme, to eliminate the selectable markers while leaving the XCRF sequences of interest that have been inserted by an homologous recombination event. Again, two selectable markers are needed: a positive selection marker to select for

the recombination event and a negative selection marker to select for the homologous recombination event. Vectors and methods using the Cre-*lox*P system are described by Zou *et al.* (1994), which disclosure is hereby incorporated by reference in its entirety.

Thus, a second preferred DNA construct of the invention comprises, from 5'-end to 3'-end: (a) a first nucleotide sequence that is comprised in the XCRF genomic sequence; (b) a nucleotide sequence comprising a polynucleotide encoding a positive selection marker, said nucleotide sequence comprising additionally two sequences defining a site recognized by a recombinase, such as a *lox*P site, the two sites being placed in the same orientation; and (c) a second nucleotide sequence that is comprised in the XCRF genomic sequence, and is located on the genome downstream of the first XCRF nucleotide sequence (a). Preferably the nucleotide sequences (a) and (c) comprise all or part of SEQ ID NO: 1, 3, 5 or 7.

The sequences defining a site recognized by a recombinase, such as a *lox*P site, are preferably located within the nucleotide sequence (b) at suitable locations bordering the nucleotide sequence for which the conditional excision is sought. In one specific embodiment, two *lox*P sites are located at each side of the positive selection marker sequence, in order to allow its excision at a desired time after the occurrence of the homologous recombination event.

In a preferred embodiment of a method using the third DNA construct described above, the excision of the polynucleotide fragment bordered by the two sites recognized by a recombinase, preferably two loxP sites, is performed at a desired time, due to the presence within the genome of the recombinant host cell of a sequence encoding the Cre enzyme operably linked to a promoter sequence, preferably an inducible promoter, more preferably a tissue-specific promoter sequence and most preferably a promoter sequence which is both inducible and tissue-specific, such as described by Gu *et al.* (1994).

The presence of the Cre enzyme within the genome of the recombinant cell host may result from the breeding of two transgenic animals, the first transgenic animal bearing the XCRF-derived sequence of interest containing the *lox*P sites as described above and the second transgenic animal bearing the *Cre* coding sequence operably linked to a suitable promoter sequence, such as described by Gu *et al.* (1994).

Spatio-temporal control of the Cre enzyme expression may also be achieved with an adenovirus based vector that contains the Cre gene thus allowing infection of cells, or *in vivo* infection of organs, for delivery of the Cre enzyme, such as described by Anton and Graham (1995) and Kanegae *et al.* (1995), which disclosures are hereby incorporated by reference in their entireties.

The DNA constructs described above may be used to introduce a desired nucleotide sequence of the invention, preferably an XCRF genomic sequence or an XCRF cDNA sequence, and most preferably

an altered copy of an XCRF genomic or cDNA sequence, within a predetermined location of the targeted genome, leading either to the generation of an altered copy of a targeted gene (knock-out homologous recombination) or to the replacement of a copy of the targeted gene by another copy sufficiently homologous to allow an homologous recombination event to occur (knock-in homologous recombination).

VI. Transgenic animals

The present invention also provides methods and compositions for the generation of non-human animals and plants that express the recombinant XCRF polypeptides, of the present invention. The animals or plants can be transgenic, i.e. each of their cells contains a gene encoding an XCRF polypeptide, or, alternatively, a polynucleotide encoding an XCRF polypeptide can be introduced into somatic cells of the animal or plant, e.g. into mammary secretory epithelial cells of a mammal. In preferred embodiments, the non-human animal is a mammal such as a cow, sheep, goat, pig, or rabbit.

Methods of making transgenic animals such as mammals are well known to those of skill in the art, and any such method can be used in the present invention. Briefly, transgenic mammals can be produced, e.g., by transfecting a pluripotential stem cell such as an ES cell with a polynucleotide encoding a polypeptide of interest. Successfully transformed ES cells can then be introduced into an early stage embryo that is then implanted into the uterus of a mammal of the same species. In certain cases, the transformed ("transgenic") cells will comprise part of the germ line of the resulting animal, and adult animals comprising the transgenic cells in the germ line can then be mated to other animals, thereby eventually producing a population of transgenic animals that have the transgene in each of their cells, and which can stably transmit the transgene to each of their offspring. Other methods of introducing the polynucleotide can be used, for example introducing the polynucleotide encoding the polypeptide of interest into a fertilized egg or early stage embryo via microinjection. Alternatively, the transgene may be introduced into an animal by infection of zygotes with a retrovirus containing the transgene (Jaenisch, R. (1976) Proc. Natl. Acad. Sci. USA 73, 1260-1264). Methods of making transgenic mammals are described, e.g., in Wall et al. (1992) J Cell Biochem 1992 49:113-20; Hogan, et al. (1986) in Manipulating the mouse embryo. A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.; in WO 91/08216, or in U.S. Patent No. 4,736,866.

In a preferred method, the polynucleotides are microinjected into the fertilized oocyte. Typically, fertilized oocytes are microinjected using standard techniques, and then cultured in vitrountil a "pre-implantation embryo" is obtained. Such pre-implantation embryos preferably contain approximately 16 to 150 cells. Methods for culturing fertilized oocytes to the pre-implantation stage are described, e.g., by Gordon et al. ((1984) Methods in Enzymology, 101, 414); Hogan et al. ((1986) in

Manipulating the mouse embryo. A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y) (for the mouse embryo); Hammer et al. ((1985) Nature, 315, 680) (for rabbit and porcine embryos); Gandolfi et al. ((1987) J. Reprod. Fert. 81, 23-28); Rexroad et al. ((1988) J. Anim. Sci. 66, 947-953) (for ovine embryos); and Eyestone et al. ((1989) J. Reprod. Fert. 85, 715-720); Camous et al. ((1984) J. Reprod. Fert. 72, 779-785); and Heyman et al. ((1987) Theriogenology 27, 5968) (for bovine embryos); the disclosures of each of which are incorporated herein in their entireties. Pre-implantation embryos are then transferred to an appropriate female by standard methods to permit the birth of a transgenic or chimeric animal, depending upon the stage of development when the transgene is introduced.

As the frequency of transgene incorporation is often low, the detection of transgene integration in pre-implantation embryos is often desirable using any of the herein-described methods. Any of a number of methods can be used to detect the presence of a transgene in a pre-implantation embryo. For example, one or more cells may be removed from the pre-implantation embryo, and the presence or absence of the transgene in the removed cell or cells can be detected using any standard method e.g. PCR. Alternatively, the presence of a transgene can be detected in utero or post partum using standard methods.

In a particularly preferred embodiment of the present invention, transgenic mammals are generated that secrete recombinant XCRF polypeptides in their milk. As the mammary gland is a highly efficient protein-producing organ, such methods can be used to produce protein concentrations in the gram per liter range, and often significantly more. Preferably, expression in the mammary gland is accomplished by operably linking the polynucleotide encoding the XCRF polypeptide to a mammary gland specific promoter and, optionally, other regulatory elements. Suitable promoters and other elements include, but are not limited to, those derived from mammalian short and long WAP, alpha, beta, and kappa, casein, alpha and beta lactoglobulin, beta-CN 5' genes, as well as the the mouse mammary tumor virus (MMTV) promoter. Such promoters and other elements may be derived from any mammal, including, but not limited to, cows, goats, sheep, pigs, mice, rabbits, and guinea pigs. Promoter and other regulatory sequences, vectors, and other relevant teachings are provided, e.g., by Clark (1998) J Mammary Gland Biol Neoplasia 3:337-50; Jost et al. (1999) Nat Biotechnol 17:160-4; U.S. Patent Nos. 5,994,616; 6,140,552; 6,013,857; Sohn et al. (1999) DNA Cell Biol. 18:845-52; Kim et al. (1999) J Biochem (Japan) 126:320-5; Soulier et al. (1999) Euro J Biochem 260:533-9; Zhang et al. (1997) Chin J Biotech 13:271-6; Rijnkels et al. (1998) Transgen Res 7:5-14; Korhonen et al. (1997) Euro J Biochem 245:482-9; Uusi-Oukari et al. (1997) Transgen Res 6:75-84; Hitchin et al. (1996) Prot Expr Purif 7:247-52; Platenburg et al. (1994) Transgen Res 3:99-108; Heng-Cherl et al. (1993) Animal

Biotech. 4:89-107; and Christa et al. (2000) Euro J Biochem 267:1665-71; the entire disclosures of each of which is herein incorporated by reference.

In another embodiment, the polypeptides of the invention can be produced in milk by introducing polynucleotides encoding the polypeptides into somatic cells of the mammary gland in vivo, e.g. mammary secreting epithelial cells. For example, plasmid DNA can be infused through the nipple canal, e.g. in association with DEAE-dextran (see, e.g., Hens et al. (2000) Biochim. Biophys. Acta 1523:161-171), in association with a ligand that can lead to receptor-mediated endocytosis of the construct (see, e.g., Sobolev et al. (1998) 273:7928-33), or in a viral vector such as a retroviral vector, e.g. the Gibbon ape leukemia virus (see, e.g., Archer et al. (1994) PNAS 91:6840-6844). In any of these embodiments, the polynucleotide may be operably linked to a mammary gland specific promoter, as described above, or, alternatively, any strongly expressing promoter such as CMV or MoMLV LTR.

The suitability of any vector, promoter, regulatory element, etc. for use in the present invention can be assessed beforehand by transfecting cells such as mammary epithelial cells, e.g. MacT cells (bovine mammary epithelial cells) or GME cells (goat mammary epithelial cells), in vitro and assessing the efficiency of transfection and expression of the transgene in the cells.

For *in vivo* administration, the polynucleotides can be administered in any suitable formulation, at any of a range of concentrations (e.g. 1-500 μg/ml, preferably 50-100 μg/ml), at any volume (e.g. 1-100 ml, preferably 1 to 20 ml), and can be administered any number of times (e.g. 1, 2, 3, 5, or 10 times), at any frequency (e.g. every 1, 2, 3, 5, 10, or any number of days). Suitable concentrations, frequencies, modes of administration, etc. will depend upon the particular polynucleotide, vector, animal, etc., and can readily be determined by one of skill in the art.

In a preferred embodiment, a retroviral vector such as as Gibbon ape leukemia viral vector is used, as described in Archer et al. ((1994) PNAS 91:6840-6844). As retroviral infection typically requires cell division, cell division in the mammary glands can be stimulated in conjunction with the administration of the vector, e.g. using a factor such as estrodiol benzoate, progesterone, reserpine, or dexamethasone. Further, retroviral and other methods of infection can be facilitated using accessory compounds such as polybrene.

In any of the herein-described methods for obtaining XCRF polypeptides from milk, the quantity of milk obtained, and thus the quantity of XCRF polypeptides produced, can be enhanced using any standard method of lactation induction, e.g. using hexestrol, estrogen, and/or progesterone.

The polynucleotides used in such embodiments can either encode a full-length XCRF polypeptide or an XCRF polypeptide fragment. Typically, the encoded polypeptide will include a signal sequence to ensure the secretion of the protein into the milk. Where a full length XCRF sequence is used, the full-length protein can, e.g., be isolated from milk and cleaved *in vitro* using a suitable

protease. Alternatively, a second, protease-encoding polynucleotide can be introduced into the animal or into the mammary gland cells, whereby expression of the protease results in the cleavage of the XCRF polypeptide *in vivo*, thereby allowing the direct isolation of XCRF fragments from milk.

VII. Pharmaceutical or Physiologically Acceptable Compositions of the Invention

The XCRF polypeptides of the invention can be administered to non-human animals and/or humans, alone or in pharmaceutical or physiologically acceptable compositions where they are mixed with suitable carriers or excipient(s). The pharmaceutical or physiologically acceptable composition is then provided at a therapeutically effective dose. A therapeutically effective dose refers to that amount of an XCRF polypeptide sufficient to result in prevention or amelioration of symptoms or physiological status of metabolic-related diseases or disorders as determined by the methods described herein. A therapeutically effective dose can also refer to the amount of an XCRF polypeptide necessary for a reduction in weight or a prevention of an increase in weight or prevention of an increase in the rate of weight gain in persons desiring this affect for cosmetic reasons. A therapeutically effective dosage of an XCRF polypeptide of the invention is that dosage that is adequate to promote weight loss or weight gain with continued periodic use or administration. Techniques for formulation and administration of XCRF polypeptides may be found in "Remington's Pharmaceutical Sciences," Mack Publishing Co., Easton, PA, latest edition.

Other diseases or disorders that XCRF polypeptides of the invention could be used to treat or prevent include, but are not limited to, obesity and obesity-related diseases and disorders such as obesity, impaired glucose tolerance, insulin resistance, atherosclerosis, atheromatous disease, heart disease, hypertension, stroke, Syndrome X, Non-Insulin Dependent Diabetes Mellitus (NIDDM, or Type II diabetes) and Insulin Dependent Diabetes Mellitus (IDDM or Type I diabetes). Diabetes-related complications to be treated by the methods of the invention include microangiopathic lesions, ocular lesions, retinopathy, neuropathy, renal lesions. Heart disease includes, but is not limited to, cardiac insufficiency, coronary insufficiency, and high blood pressure. Other obesity-related disorders to be treated by compounds of the invention include hyperlipidemia and hyperuricemia. Yet other obesity-related diseases or disorders of the invention include cachexia, wasting, AIDS-related weight loss, cancer-related weight loss, anorexia, and bulimia. The XCRF polypeptides may also be used to enhance physical performance during work or exercise or enhance a feeling of general well-being. Physical performance activities include walking, running, jumping, lifting and/or climbing.

The XCRF polypeptides or antagonists thereof may also be used to treat dyslexia, attention-deficit disorder (ADD), attention-deficit/hyperactivity disorder (ADHD), and psychiatric disorders such

as schizophrenia by modulating fatty acid metabolism, more specifically, the production of certain long-chain polyunsaturated fatty acids.

It is expressly considered that the XCRF polypeptides of the invention may be provided alone or in combination with other pharmaceutically or physiologically acceptable compounds. Other compounds useful for the treatment of obesity and other diseases and disorders are currently well-known in the art.

In a preferred embodiment, the XCRF polypeptides are useful for, and used in, the treatment of insulin resistance and diabetes using methods described herein and known in the art. More particularly, a preferred embodiments relates to process for the therapeutic modification and regulation of glucose metabolism in an animal or human subject, which comprises administering to a subject in need of treatment (alternatively on a timed daily basis) XCRF polypeptide (or polynucleotide encoding said polypeptide) in dosage amount and for a period sufficient to reduce plasma glucose levels in said animal or human subject.

Further preferred embodiments relate to methods for the prophylaxis or treatment of diabetes comprising administering to a subject in need of treatment (alternatively on a timed daily basis) an XCRF polypeptide (or polynucleotide encoding said polypeptide) in dosage amount and for a period sufficient to reduce plasma glucose levels in said animal or human subject.

Routes of Administration.

Suitable routes of administration include oral, nasal, rectal, transmucosal, or intestinal administration, parenteral delivery, including intramuscular, subcutaneous, intramedullary injections, as well as intrathecal, direct intraventricular, intravenous, intraperitoneal, intranasal, intrapulmonary (inhaled) or intraocular injections using methods known in the art. A particularly useful method of administering compounds for promoting weight loss involves surgical implantation, for example into the abdominal cavity of the recipient, of a device for delivering XCRF polypeptides over an extended period of time. Other particularly preferred routes of administration are aerosol and depot formulation. Sustained release formulations, particularly depot, of the invented medicaments are expressly contemplated.

Composition/Formulation

Pharmaceutical or physiologically acceptable compositions and medicaments for use in accordance with the present invention may be formulated in a conventional manner using one or more physiologically acceptable carriers comprising excipients and auxiliaries. Proper formulation is dependent upon the route of administration chosen.

Certain of the medicaments described herein will include a pharmaceutically or physiologically acceptable acceptable carrier and at least one polypeptide that is an XCRF polypeptide of the invention. For injection, the agents of the invention may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks's solution, Ringer's solution, or physiological saline buffer such as a phosphate or bicarbonate buffer. For transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

Pharmaceutical or physiologically acceptable preparations that can be taken orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules can contain the active ingredients in admixture with fillers such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers may be added. All formulations for oral administration should be in dosages suitable for such administration.

For buccal administration, the compositions may take the form of tablets or lozenges formulated in conventional manner.

For administration by inhalation, the compounds for use according to the present invention are conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebulizer, with the use of a suitable gaseous propellant, *e.g.,* carbon dioxide. In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of, *e.g.,* gelatin, for use in an inhaler or insufflator, may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

The compounds may be formulated for parenteral administration by injection, *e.g.,* by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, *e.g.,* in ampoules or in multi-dose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents.

Pharmaceutical or physiologically acceptable formulations for parenteral administration include aqueous solutions of the active compounds in water-soluble form. Aqueous suspensions may contain substances that increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Optionally, the suspension may also contain suitable stabilizers or agents that increase the solubility of the compounds to allow for the preparation of highly concentrated solutions.

Alternatively, the active ingredient may be in powder or lyophilized form for constitution with a suitable vehicle, such as sterile pyrogen-free water, before use.

In addition to the formulations described previously, the compounds may also be formulated as a depot preparation. Such long acting formulations may be administered by implantation (for example subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, the compounds may be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

Additionally, the compounds may be delivered using a sustained-release system, such as semipermeable matrices of solid hydrophobic polymers containing the therapeutic agent. Various sustained release materials have been established and are well known by those skilled in the art. Sustained-release capsules may, depending on their chemical nature, release the compounds for a few weeks up to over 100 days.

Depending on the chemical nature and the biological stability of the therapeutic reagent, additional strategies for protein stabilization may be employed.

The pharmaceutical or physiologically acceptable compositions also may comprise suitable solid or gel phase carriers or excipients. Examples of such carriers or excipients include but are not limited to calcium carbonate, calcium phosphate, various sugars, starches, cellulose derivatives, gelatin, and polymers such as polyethylene glycols.

Effective Dosage.

Pharmaceutical or physiologically acceptable compositions suitable for use in the present invention include compositions wherein the active ingredients are contained in an effective amount to achieve their intended purpose. More specifically, a therapeutically effective amount means an amount effective to prevent development of or to alleviate the existing symptoms of the subject being treated. Determination of the effective amounts is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein.

For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. For example, a dose can be formulated in animal models to achieve a circulating concentration range that includes or encompasses a concentration point or range shown to increase leptin or lipoprotein uptake or binding in an *in vitro* system. Such information can be used to more accurately determine useful doses in humans.

A therapeutically effective dose refers to that amount of the compound that results in amelioration of symptoms in a patient. Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.,* for determining the LD50, (the dose lethal to 50% of the test population) and the ED50 (the dose therapeutically effective in

50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio between LD5O and ED5O. Compounds that exhibit high therapeutic indices are preferred.

The data obtained from these cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED50, with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition. *(See, e.g.,* Fingl et al., 1975, in "The Pharmacological Basis of Therapeutics", Ch. 1).

Dosage amount and interval may be adjusted individually to provide plasma levels of the active compound which are sufficient to maintain or prevent weight loss or gain, depending on the particular situation. Dosages necessary to achieve these effects will depend on individual characteristics and route of administration.

Dosage intervals can also be determined using the value for the minimum effective concentration. Compounds should be administered using a regimen that maintains plasma levels above the minimum effective concentration for 10-90% of the time, preferably between 30-90%; and most preferably between 50-90%. In cases of local administration or selective uptake, the effective local concentration of the drug may not be related to plasma concentration.

The amount of composition administered will, of course, be dependent on the subject being treated, on the subject's weight, the severity of the affliction, the manner of administration and the judgment of the prescribing physician.

A preferred dosage range for the amount of an XCRF polypeptide of the invention, which can be administered on a daily or regular basis to achieve desired results, including a reduction in levels of circulating plasma triglyceride-rich lipoproteins, range from 0.05 – 1.0 mg/kg body mass. A more preferred dosage range is from 0.1 – 5 mg/kg. A more preferred dose is 0.25 – 2.5 mg/kg. Of course, these daily dosages can be delivered or administered in small amounts periodically during the course of a day. It is noted that these dosage ranges are only preferred ranges and are not meant to be limiting to the invention.

VIII. <u>Methods of Treatment</u>

The invention is drawn *inter alia* to methods of preventing or treating metabolic-related diseases and disorders comprising providing an individual in need of such treatment with an XCRF polypeptide of the invention. Preferably, the XCRF polypeptide has metabolic-related activity either *in vitro* or *in vivo*. Preferably the XCRF polypeptide is provided to the individual in a

pharmaceutical composition that is preferably taken orally. Preferably the individual is a mammal, and most preferably a human. In preferred embodiments, the metabolic-related disease or disorder is selected from the group consisting of atherosclerosis, cardiovascular disease, impaired glucose tolerance, insulin resistance, hypertension, stroke, Syndrome X, Type I diabetes, Type II diabetes and lipoatrophic diabetes. Diabetes-related complications to be treated by the methods of the invention include microangiopathic lesions, ocular lesions, retinopathy, neuropathy and renal lesions. Heart disease includes, but is not limited to, cardiac insufficiency, coronary insufficiency, and high blood pressure. Other metabolic-related disorders to be treated by compounds of the invention include hyperlipidemia, hypertriglyceridemia, and hyperuricemia. Yet other metabolic-related diseases or disorders of the invention include cachexia, wasting, AIDS-related weight loss, cancer-related weight loss, neoplasia-related weight loss, anorexia, and bulimia. In preferred embodiments, XCRF polypeptides in pharmaceutical compositions are used to modulate body weight in healthy individuals for cosmetic reasons.

The invention also features a method of preventing or treating metabolic-related diseases and disorders comprising providing an individual in need of such treatment with a compound identified by assays of the invention (described in Section VI of the Preferred Embodiments of the Invention and in the Examples). Preferably these compounds antagonize or agonize effects of XCRF polypeptides in cells *in vitro*, muscles *ex vivo,* or in animal models. Alternatively, these compounds agonize or antagonize the effects of XCRF polypeptides on leptin and/or lipoprotein uptake and/or binding. Optionally, these compounds prevent the interaction, binding, or uptake of XCRF polypeptides with LSR *in vitro* or *in vivo.* Preferably, the compound is provided to the individual in a pharmaceutical composition that is preferably taken orally. Preferably the individual is a mammal, and most preferably a human. In preferred embodiments, the metabolic-related disease or disorder is selected from the group consisting of obesity and metabolic-related diseases and disorders such as atherosclerosis, heart disease, insulin resistance, hypertension, stroke, Syndrome X, Type I diabetes, Type II diabetes, and lipoatrophic diabetes. Diabetes-related complications to be treated by the methods of the invention include microangiopathic lesions, ocular lesions, retinopathy, neuropathy and renal lesions. Heart disease includes, but is not limited to, cardiac insufficiency, coronary insufficiency, and high blood pressure. Other metabolic-related disorders to be treated by compounds of the invention include hyperlipidemia, hypertriglyceridemia, and hyperuricemia.

In further preferred embodiments, the present invention of said pharmaceutical or physiologically acceptable composition can be used as a method to control blood glucose in some individuals, particularly those with Type I diabetes, Type II diabetes, or insulin resistance, in combination with insulin therapy.

In further preferred embodiments, the present invention of said pharmaceutical or physiologically acceptable composition can be used as a method to control body weight in some individuals, particularly those with Type I diabetes, Type II diabetes, or insulin resistance, in combination with insulin therapy.

In further preferred embodiments, the present invention of said pharmaceutical or physiologically acceptable composition can be used as a method to control blood glucose in some individuals, particularly those with Type I diabetes, Type II diabetes, or insulin resistance, alone, without combination of insulin therapy.

In further preferred embodiments, the present invention of said pharmaceutical or physiologically acceptable composition can be used as a method to control body weight in some individuals, particularly those with Type II diabetes or insulin resistance, alone, without combination of insulin therapy. In still a further preferred embodiment, the control of body weight is due in part or in whole to a decrease in mass of 1) subcutaneous adipose tissue and/or 2) visceral (omental) adipose tissue.

In further preferred embodiments, the present invention of said pharmaceutical or physiologically acceptable composition can be used as a method of preventing weight gain in some individuals, particularly those with Type I diabetes, Type II diabetes, or insulin resistance or alternatively for cosmetic reasons.

In further preferred embodiments, the present invention of said pharmaceutical or physiologically acceptable composition can be used as a method of reducing weight in some individuals, particularly those with Type I diabetes, Type II diabetes, or insulin resistance or alternatively for cosmetic reasons.

In further preferred embodiments, the present invention of said pharmaceutical or physiologically acceptable composition can be used as a method of maintaining weight loss in some individuals, particularly those with Type I diabetes, Type II diabetes, or insulin resistance or alternatively for cosmetic reasons.

In a further preferred embodiment, the present invention may be used in complementary therapy, particularly in some individuals, particularly those with Type I diabetes, Type II diabetes, or insulin resistance, to improve their weight or glucose control in combination with an insulin secretagogue or an insulin sensitising agent. Preferably, the insulin secretagogue is 1,1-dimethyl-2-(2-morpholino phenyl)guanidine fumarate (BTS67582) or a sulphonylurea selected from tolbutamide, tolazamide, chlorpropamide, glibenclamide, glimepiride, glipizide and glidazide. Preferably, the insulin sensitising agent is selected from metformin, ciglitazone, troglitazone and pioglitazone.

The present invention further provides a method of improving the body weight or glucose control of some individuals, particularly those with Type I diabetes, Type II diabetes, or insulin resistance,alone, without an insulin secretagogue or an insulin sensitising agent.

In a further preferred embodiment, the present invention may be administered either concomitantly or concurrently, with the insulin secretagogue or insulin sensitising agent for example in the form of separate dosage units to be used simultaneously, separately or sequentially (either before or after the secretagogue or either before or after the sensitising agent). Accordingly, the present invention further provides for a composition of pharmaceutical or physiologically acceptable composition and an oral insulin secretagogue or insulin sensitising agent as a combined preparation for simultaneous, separate or sequential use for the improvement of body weight or glucose control in some individuals, particularly those with Type I diabetes, Type II diabetes, or insulin resistance.

In further preferred embodiments, the present invention of said pharmaceutical or physiologically acceptable composition further provides a method for the use as an insulin sensitiser.

In further preferred embodiments, the present invention of said pharmaceutical or physiologically acceptable composition can be used as a method to improve insulin sensitivity in some individuals, particularly those with Type I diabetes, Type II diabetes, or insulin resistance, in combination with insulin therapy.

In further preferred embodiments, the present invention of said pharmaceutical or physiologically acceptable composition can be used as a method to improve insulin sensitivity in some individuals, particularly those with Type II diabetes or insulin resistance, without insulin therapy.

In further preferred embodiments, the present invention of said pharmaceutical or physiologically acceptable composition further provides a method for the use as an inhibitor of the progression from impaired glucose tolerance to insulin resistance.

IX. Ligands interacting with XCRF polypeptides

For the purpose of the present invention, a Ligand means a molecule, such as a protein, a peptide, an antibody or any synthetic chemical compound capable of binding to an XCRF protein or one of its fragments or variants or to modulate the expression of the polynucleotide coding for XCRF or a fragment or variant thereof.

In the Ligand screening method according to the present invention, a biological sample or a defined molecule to be tested as a putative Ligand of an XCRF protein is brought into contact with the corresponding purified XCRF protein, for example the corresponding purified recombinant XCRF protein produced by a recombinant cell host as described herein, in order to form a complex between this protein and the putative Ligand molecule to be tested.

As an illustrative example, to study the interaction of an XCRF protein, or a fragment comprising a contiguous span of at least 6 amino acids, preferably at least 8 to 10 amino acids, more preferably at least 12, 15, 20, 25, 30, 40, 50, or 100 amino acids of a polypeptide selected from the group consisting of SEQ ID NO: 2,. 4, 6 or 8, with drugs or small molecules, such as molecules generated through combinatorial chemistry approaches, the microdialysis coupled to HPLC method described by Wang *et al.* (1997) or the affinity capillary electrophoresis method described by Bush *et al.* (1997), the disclosures of which are incorporated by reference, can be used.

In further methods, peptides, drugs, fatty acids, lipoproteins, or small molecules which interact with an XCRF protein, or a fragment comprising a contiguous span of at least 6 amino acids, preferably at least 8 to 10 amino acids, more preferably at least 12, 15, 20, 25, 30, 40, 50, or 100 amino acids of a polypeptide selected from the group consisting of sequences of SEQ ID NO: 2. 4. 6 or 8 may be identified using assays such as the following. The molecule to be tested for binding is labelled with a detectable label, such as a fluorescent, radioactive, or enzymatic tag and placed in contact with immobilized XCRF protein, or a fragment thereof under conditions that permit specific binding to occur. After removal of non-specifically bound molecules, bound molecules are detected using appropriate means.

Various candidate substances or molecules can be assayed for interaction with an XCRF polypeptide. These substances or molecules include, without being limited to, natural or synthetic organic compounds or molecules of biological origin such as polypeptides. When the candidate substance or molecule comprises a polypeptide, this polypeptide may be the resulting expression product of a phage clone belonging to a phage-based random peptide library, or alternatively the polypeptide may be the resulting expression product of a cDNA library cloned in a vector suitable for performing a two-hybrid screening assay.

A. <u>Candidate Ligands obtained by affinity chromatography.</u>

Proteins or other molecules interacting with an XCRF protein, or a fragment thereof comprising a contiguous span of at least 6 amino acids, preferably at least 8 to 10 amino acids, more preferably at least 12, 15, 20, 25, 30, 40, 50, or 100 amino acids of a polypeptide selected from the group consisting of sequences of SEQ ID NO: 2, 4, 6 or 8, can also be found using affinity columns which contain the XCRF protein, or a fragment thereof. The XCRF protein, or a fragment thereof, may be attached to the column using conventional techniques including chemical coupling to a suitable column matrix such as agarose, Affi Gel®, or other matrices familiar to those of skill in art. In some embodiments of this method, the affinity column contains chimeric proteins in which the XCRF protein, or a fragment thereof, is fused to glutathion S transferase (GST). A mixture of cellular proteins or pool of expressed

proteins as described above is applied to the affinity column. Proteins or other molecules interacting with the XCRF protein, or a fragment thereof, attached to the column can then be isolated and analyzed on 2-D electrophoresis gel as described in Ramunsen *et al.* (1997), the disclosure of which is incorporated by reference. Alternatively, the proteins retained on the affinity column can be purified by electrophoresis-based methods and sequenced. The same method can be used to isolate antibodies, to screen phage display products, or to screen phage display human antibodies.

B. Candidate Ligands obtained by optical biosensor methods

Proteins interacting with an XCRF protein, or a fragment comprising a contiguous span of at least 6 amino acids, preferably at least 8 to 10 amino acids, more preferably at least 12, 15, 20, 25, 30, 40, 50, or 100 amino acids of a polypeptide selected from the group consisting of sequences of SEQ ID NO: 2, 4, 6 or 8, can also be screened by using an Optical Biosensor as described in Edwards and Leatherbarrow (1997) and also in Szabo *et al.* (1995), the disclosures of which are incorporated by reference. This technique permits the detection of interactions between molecules in real time, without the need of labelled molecules. This technique is based on the surface plasmon resonance (SPR) phenomenon. Briefly, the candidate Ligand molecule to be tested is attached to a surface (such as a carboxymethyl dextran matrix). A light beam is directed towards the side of the surface that does not contain the sample to be tested and is reflected by said surface. The SPR phenomenon causes a decrease in the intensity of the reflected light with a specific association of angle and wavelength. The binding of candidate Ligand molecules cause a change in the refraction index on the surface, which change is detected as a change in the SPR signal. For screening of candidate Ligand molecules or substances that are able to interact with the XCRF protein, or a fragment thereof, the XCRF protein, or a fragment thereof, is immobilized onto a surface. This surface comprises one side of a cell through which flows the candidate molecule to be assayed. The binding of the candidate molecule on the XCRF protein, or a fragment thereof, is detected as a change of the SPR signal. The candidate molecules tested may be proteins, peptides, carbohydrates, lipids, or small molecules generated by combinatorial chemistry. This technique may also be performed by immobilizing eukaryotic or prokaryotic cells or lipid vesicles exhibiting an endogenous or a recombinantly expressed XCRF protein at their surface.

The main advantage of the method is that it allows the determination of the association rate between the XCRF protein and molecules interacting with the XCRF protein. It is thus possible to select specifically Ligand molecules interacting with the XCRF protein, or a fragment thereof, through strong or conversely weak association constants.

C. Candidate Ligands obtained through a two-hybrid screening assay.

The yeast two-hybrid system is designed to study protein-protein interactions *in vivo* (Fields and Song, 1989), which disclosure is hereby incorporated by reference in its entirety, and relies upon the fusion of a bait protein to the DNA binding domain of the yeast Gal4 protein. This technique is also described in the US Patent N° US 5,667,973 and the US Patent N° 5,283,173, the technical teachings of both patents being herein incorporated by reference.

The general procedure of library screening by the two-hybrid assay may be performed as described by Harper *et al.* (1993) or as described by Cho *et al.* (1998) or also Fromont-Racine *et al.* (1997), which disclosures are hereby incorporated by reference in their entireties.

The bait protein or polypeptide comprises, consists essentially of, or consists of an XCRF polypeptide or a fragment thereof comprising a contiguous span of at least 6 amino acids, preferably at least 8 to 10 amino acids, more preferably at least 12, 15, 20, 25, 30, 40, 50, or 100 amino acids of a polypeptide selected from the group consisting of sequences of SEQ ID NO: 2, 4, 6 or 8.

More precisely, the nucleotide sequence encoding the XCRF polypeptide or a fragment or variant thereof is fused to a polynucleotide encoding the DNA binding domain of the GAL4 protein, the fused nucleotide sequence being inserted in a suitable expression vector, for example pAS2 or pM3.

Then, a human cDNA library is constructed in a specially designed vector, such that the human cDNA insert is fused to a nucleotide sequence in the vector that encodes the transcriptional domain of the GAL4 protein. Preferably, the vector used is the pACT vector. The polypeptides encoded by the nucleotide inserts of the human cDNA library are termed "prey" polypeptides.

A third vector contains a detectable marker gene, such as beta galactosidase gene or CAT gene that is placed under the control of a regulation sequence that is responsive to the binding of a complete Gal4 protein containing both the transcriptional activation domain and the DNA binding domain. For example, the vector pG5EC may be used.

Two different yeast strains are also used. As an illustrative but non-limiting example the two different yeast strains may be the following:

Y190, the phenotype of which is (MATa, Leu2-3, 112 ura3-12, trp1-901, his3-D200, ade2-101, gal4Dgal180D URA3 GAL-LacZ, LYS GAL-HIS3, cyh^r);

Y187, the phenotype of which is (MATa gal4 gal80 his3 trp1-901 ade2-101 ura3-52 leu2-3, -112 URA3 GAL-lacZmet$^-$), which is the opposite mating type of Y190.

Briefly, 20 μg of pAS2/XCRF and 20 μg of pACT-cDNA library are co-transformed into yeast strain Y190. The transformants are selected for growth on minimal media lacking histidine, leucine and tryptophan, but containing the histidine synthesis inhibitor 3-AT (50 mM). Positive colonies are

screened for beta galactosidase by filter lift assay. The double positive colonies (His$^+$, beta-gal$^+$) are then grown on plates lacking histidine, leucine, but containing tryptophan and cycloheximide (10 mg/ml) to select for loss of pAS2/XCRF plasmid but retention of pACT-cDNA library plasmids. The resulting Y190 strains are mated with Y187 strains expressing XCRFor non-related control proteins; such as cyclophilin B, lamin, or SNF1, as Gal4 fusions as described by Harper *et al.* (1993) and by Bram *et al.* (1993), which disclosures are hereby incorporated by reference in their entireties, and screened for beta galactosidase by filter lift assay. Yeast clones that are beta gal- after mating with the control Gal4 fusions are considered false positives.

In another embodiment of the two-hybrid method according to the invention, interaction between the XCRF or a fragment or variant thereof with cellular proteins may be assessed using the Matchmaker Two Hybrid System 2 (Catalog No. K1604-1, Clontech). As described in the manual accompanying the kit, the disclosure of which is incorporated herein by reference, nucleic acids encoding the XCRF protein or a portion thereof, are inserted into an expression vector such that they are in frame with DNA encoding the DNA binding domain of the yeast transcriptional activator GAL4. A desired cDNA, preferably human cDNA, is inserted into a second expression vector such that they are in frame with DNA encoding the activation domain of GAL4. The two expression plasmids are transformed into yeast and the yeast are plated on selection medium which selects for expression of selectable markers on each of the expression vectors as well as GAL4 dependent expression of the HIS3 gene. Transformants capable of growing on medium lacking histidine are screened for GAL4 dependent lacZ expression. Those cells that are positive in both the histidine selection and the lacZ assay contain interaction between XCRF and the protein or peptide encoded by the initially selected cDNA insert.

X. Assays for Identifying Modulators of XCRF Polypeptide Activity

The invention features methods of screening for one or more compounds that modulate the activity of XCRF in cells, which includes providing potential compounds to be tested to the cells,. Exempiary assays that may be used are described in the Examples section. To these assays would be added compounds to be tested for their inhibitory or stimulatory activity as compared to the effects of XCRF polypeptides alone. Other assays in which an effect is observed based on the addition of XCRF polypeptides can also be used to screen for modulators of XCRF polypeptide activity or effects of the presence of XCRF polypeptides on cells. The essential step is to apply an unknown compound and then to monitor an assay for a change from what is seen when only XCRF polypeptides are applied to the cell. A change is defined as something that is significantly different in the presence of the compound plus XCRF polypeptide compared to XCRF polypeptide alone. In this case, significantly different

would be an "increase" or a "decrease" in a measurable effect of at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, or 75%.

The term "modulation" as used herein refers to a measurable change in an activity. Examples include, but are not limited to, lipolysis stimulated receptor (LSR) modulation, leptin modulation, lipoprotein modulation, plasma FFA levels, FFA oxidation, TG levels, glucose levels, and weight. These effects can be *in vitro* or preferably *in vivo.* Modulation of an activity can be either an increase or a decrease in the activity. Thus, LSR activity can be increased or decreased, leptin activity can be increased or decreased, and lipoprotein activity can be increased or decreased. Similarly, FFA, TG, glucose levels and weight can be increased or decreased *in vivo* Free Fatty Acid oxidation can be increased or decreased *in vivo* or *ex vivo.*

By "LSR" activity is meant expression of LSR on the surface of the cell, or in a particular conformation, as well as its ability to bind, uptake, and degrade leptin and lipoprotein. By "leptin" activity is meant its binding, uptake and degradation by LSR, as well as its transport across a blood brain barrier, and potentially these occurrences where LSR is not necessarily the mediating factor or the only mediating factor. Similarly, by "lipoprotein" activity is meant its binding, uptake and degradation by LSR, as well as these occurrences where LSR is not necessarily the mediating factor or the only mediating factor. Exemplary assays are provided in the Examples. These assay and other comparable assays can be used to determine/identify compounds that modulate XCRF polypeptide activity. In some cases it may be important to identify compounds that modulate some but not all of the XCRF polypeptide activities, although preferably all activities are modified.

The term "increasing" as used herein refers to the ability of a compound to increase the activity of XCRF polypeptides in some measurable way compared to the effect of XCRF polypeptides in its absence. As a result of the presence of the compound leptin binding and/or uptake might increase, for example, as compared to controls in the presence of the XCRF polypeptide alone. Preferably, an increase in activity is at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, or 75% compared to the level of activity in the presence of the XCRF polypeptide. Preferably said XCRF polypeptide is XCRF polypeptide fragment comprising all or part of the globular C-terminal C1q homology domain.

Similarly, the term "decreasing" as used herein refers to the ability of a compound to decrease an activity in some measurable way compared to the effect of an XCRF polypeptide in its absence. For example, the presence of the compound decreases the plasma concentrations of FFA, TG, and glucose in mice. Also as a result of the presence of a compound leptin binding and/or uptake might decrease, for example, as compared to controls in the presence of the XCRF polypeptides alone. Preferably, an decrease in activity is at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, or 75% as

compared to the level of activity in the presence of the XCRF polypeptides alone. Preferably said XCRF polypeptide is XCRF polypeptide fragment comprising all or part of the globular C-terminal C1q homology domain.

The invention features a method for identifying a potential compound to decrease body mass in individuals in need of decreasing body mass comprising: a) contacting a cell with an XCRF polypeptide and a candidate compound; b) detecting a result selected from the group consisting of LSR modulation, leptin modulation, increase in glucose uptake or oxidation, decrease in blood lipid or triglyceride levels, increase in lipoprotein binding, uptake or degradation; FFA oxidation increase; and c) wherein said result identifies said potential compound if said result differs from said result when said cell is contacted with the XCRF polypeptide alone.

Alternatively, the invention features a method for identifying a potential compound to increase body mass in individuals in need of increasing body mass comprising: a) contacting a cell with an XCRF polypeptide and a candidate compound; b) detecting a result selected from the group consisting of LSR modulation, leptin modulation, decrease in glucose uptake or oxidation, increase in blood lipid or triglyceride levels, decrease in lipoprotein binding, uptake or degradation; FFA oxidation decrease; and c) wherein said result identifies said potential compound if said result differs from said result when said cell is contacted with the XCRF polypeptide alone.

In still other preferred embodiments, said potential compound is selected from the group consisting of peptides, peptide libraries, non-peptide libraries, peptoids, fatty acids, lipoproteins, medicaments, antibodies, small molecules, proteases and protease inhibitors.

XI. Assays for Identifying Antagonists of XCRF Polypeptide Activity

The invention features methods of screening compounds for one or more antagonists of XCRF polypeptide activity, wherein said activity is selected from but not restricted to lipid partitioning, lipid metabolism, and insulin-like activity. The invention further features methods of screening compounds for one or more antagonists of XCRF polypeptide activity, wherein said activity is selected from but not restricted to prevention of weight gain, weight reduction, and maintenance of weight loss. Preferred said compound is selected from but is not restricted to small molecular weight organic or inorganic compound, protein, peptide, carbohydrate, or lipid. Preferred said XCRF polypeptide having activity comprises, consists essentially of, or consists of at least 6 consecutive amino acids and not more than 258 consecutive amino acids of SEQ ID NO: 2 or 287 consecutive amino acids of SEQ ID NO: 4 or 238 consecutive amino acids of SEQ ID NO: 6 or 287 consecutive amino acids of SEQ ID NO: 8. Particularly preferred said XCRF polypeptide having activity is XCRF polypeptide fragment.

Particularly preferred said XCRF1 polypeptide fragment having activity is XCRF1 polypeptide fragment comprising all or part of the globular C-terminal C1q homology domain. More particularly preferred said XCRF1 polypeptide fragment having activity and comprising all or part of the globular C-terminal C1q homology domain is selected from amino acids 17-258, 30-258, 39-258, 46-258, 72-258, 75-258, 78-258, 99-258, 100-258, 105-258, 110-258, 115-258, 118-258, 119-258, 120-258, 121-258, 122-258, 123-258, 124-258, 125-258, 126-258, 127-258, 128-258, 129-258 or 130-258 of SEQ ID NO: 2.

Preferred said XCRF3 polypeptide having activity comprises, consists essentially of, or consists of at least 6 consecutive amino acids and not more than 287 consecutive amino acids of SEQ ID NO: 4. Particularly preferred said XCRF3 polypeptide having activity is XCRF3 polypeptide fragment. Particularly preferred said XCRF3 polypeptide fragment having activity is XCRF3 polypeptide fragment comprising all or part of the globular C-terminal C1q homology domain. More particularly preferred said XCRF3 polypeptide fragment having activity and comprising all or part of the globular C-terminal C1q homology domain is selected from amino acids 22-287, 123-287, 124-287, 125-287, 126-287, 127-287, 128-287, 129-287, 130-287, 131-287, 132-287, 133-287, 134-287, 135-287, 136-287, 137-287, 138-287, 139-287, 140-287, 141-287, 142-287, 143-287, 144-287, 145-287, 146-287, 147-287, 148-287, 149-287, 150-287, 151-287, 152-287 or 153-287 of SEQ ID NO: 4, where it is understood that amino acid 22 is taken to represent the N-terminal amino acid of mature XCRF3 polypeptide minus the signal peptide.

Preferred said XCRF4 polypeptide having activity comprises, consists essentially of, or consists of at least 6 consecutive amino acids and not more than 238 consecutive amino acids of SEQ ID NO: 6. Particularly preferred said XCRF4 polypeptide having activity is XCRF4 polypeptide fragment. Particularly preferred said XCRF4 polypeptide fragment having activity is XCRF4 polypeptide fragment comprising all or part of the globular C-terminal C1q homology domain. More particularly preferred said XCRF4 polypeptide fragment having activity and comprising all or part of the globular C-terminal C1q homology domain is selected from amino acids 16-238, 97-238, 98-238, 99-238, 100-238, 101-238, 102-238, 103-238, 104-238, 105-238, 106-238, 107-238, 108-238, 109-238 or 110-238 of SEQ ID NO: 6, where it is understood that amino acid 16 is taken to represent the N-terminal amino acid of mature XCRF4 polypeptide minus the signal peptide.

Preferred said XCRF5 polypeptide having activity comprises, consists essentially of, or consists of at least 6 consecutive amino acids and not more than 287 consecutive amino acids of SEQ ID NO: 8. Particularly preferred said XCRF5 polypeptide having activity is XCRF5 polypeptide fragment. Particularly preferred said XCRF5 polypeptide fragment having activity is XCRF5 polypeptide fragment comprising all or part of the globular C-terminal C1q homology domain. More particularly

preferred said XCRF5 polypeptide fragment having activity and comprising all or part of the globular C-terminal C1q homology domain is selected from amino acids 22-287, 123-287, 124-287, 125-287, 126-287, 127-287, 128-287, 129-287, 130-287, 131-287, 132-287, 133-287, 134-287, 135-287, 136-287, 137-287, 138-287, 139-287, 140-287, 141-287, 142-287, 143-287, 144-287, 145-287, 146-287, 147-287, 148-287, 149-287, 150-287, 151-287, 152-287 or 153-287 of SEQ ID NO: 8, where it is understood that amino acid 22 is taken to represent the N-terminal amino acid of mature XCRF5 polypeptide minus the signal peptide.

The invention further features methods of screening compounds for said antagonist of XCRF polypeptide activity comprising: a) contacting said XCRF polypeptide with or without said compound; b) detecting a result on the basis of activity, wherein said activity is selected from but not restricted to lipid partitioning, lipid metabolism, insulin-like activity, prevention of weight gain, weight reduction, and maintenance of weight loss; and c) wherein said result identifies said compound as an antagonist of XCRF polypeptide activity if said result with compound differs from said result without compound. Exemplary assays that may be used are described in Examples.

XII. Epitopes and Antibody Fusions

A preferred embodiment of the present invention is directed to eiptope-bearing polypeptides and epitope-bearing polypeptide fragments. These epitopes may be "antigenic epitopes" or both an "antigenic epitope" and an "immunogenic epitope". An "immunogenic epitope" is defined as a part of a protein that elicits an antibody response *in vivo* when the polypeptide is the immunogen. On the other hand, a region of polypeptide to which an antibody binds is defined as an "antigenic determinant" or "antigenic epitope." The number of immunogenic epitopes of a protein generally is less than the number of antigenic epitopes. See, e.g., Geysen, et al. (1983) Proc Natl Acad Sci USA 81:39984002. It is particularly noted that although a particular epitope may not be immunogenic, it is nonetheless useful since antibodies can be made *in vitro* to any epitope.

An epitope can comprise as few as 3 amino acids in a spatial conformation which is unique to the epitope. Generally an epitope consists of at least 6 such amino acids, and more often at least 8-10 such amino acids. In preferred embodiment, antigenic epitopes comprise a number of amino acids that is any integer between 3 and 50. Fragments which function as epitopes may be produced by any conventional means. See, e.g., Houghten, R. A., Proc Natl Acad Sci USA 82:5131-5135 (1985), further described in U.S. Patent No. 4,631,211. Methods for determining the amino acids which make up an immunogenic epitope include x-ray crystallography, 2-dimensional nuclear magnetic resonance, and epitope mapping, e.g., the Pepscan method described by H. Mario Geysen et al. (1984); Proc. Natl. Acad. Sci. U.S.A. 81:3998-4002; PCT Publication No. WO 84/03564; and PCT Publication No. WO

84/03506. Another example is the algorithm of Jameson and Wolf, Comp. Appl. Biosci. 4:181-186 (1988) (said references incorporated by reference in their entireties). The Jameson-Wolf antigenic analysis, for example, may be performed using the computer program PROTEAN, using default parameters (Version 4.0 Windows, DNASTAR, Inc., 1228 South Park Street Madison, WI).

The epitope-bearing fragments of the present invention preferably comprise 6 to 50 amino acids (i.e. any integer between 6 and 50, inclusive) of a polypeptide of the present invention. Also, included in the present invention are antigenic fragments between the integers of 6 and the full-length sequence of the sequence listing. All combinations of sequences between the integers of 6 and the full-length sequence of a polypeptide of the present invention are included. The epitope-bearing fragments may be specified by either the number of contiguous amino acid residues (as a sub-genus) or by specific N-terminal and C-terminal positions (as species) as described above for the polypeptide fragments of the present invention.

Antigenic epitopes are useful, for example, to raise antibodies, including monoclonal antibodies that specifically bind the epitope (See, Wilson et al., 1984; and Sutcliffe, J. G. et al., 1983). The antibodies are then used in various techniques such as diagnostic and tissue/cell identification techniques, as described herein, and in purification methods.

Similarly, immunogenic epitopes can be used to induce antibodies according to methods well known in the art (See, Sutcliffe et al., *supra*; Wilson et al., *supra*; Chow, M. et al.;(1985) and Bittle,.F. J. et al., (1985). A preferred immunogenic epitope includes the polypeptides of the sequence listing. The immunogenic epitopes may be presented together with a carrier protein, such as an albumin, to an animal system (such as rabbit or mouse) if necessary. Immunogenic epitopes comprising as few as 8 to 10 amino acids have been shown to be sufficient to raise antibodies capable of binding to, at the very least, linear epitopes in a denatured polypeptide (e.g., in Western blotting.).

Epitope-bearing polypeptides of the present invention are used to induce antibodies according to methods well known in the art including, but not limited to, *in vivo* immunization, *in vitro* immunization, and phage display methods (See, e.g., Sutcliffe, et al., *supra*; Wilson, et al., *supra*, and Bittle, et al., 1985). If *in vivo* immunization is used, animals may be immunized with free peptide; however, anti-peptide antibody titer may be boosted by coupling of the peptide to a macromolecular carrier, such as keyhole limpet hemacyanin (KLH) or tetanus toxoid. For instance, peptides containing cysteine residues may be coupled to a carrier using a linker such as -maleimidobenzoyl-N-hydroxysuccinimide ester (MBS), while other peptides may be coupled to carriers using a more general linking agent such as glutaraldehyde. Animals such as rabbits, rats and mice are immunized with either free or carrier-coupled peptides, for instance, by intraperitoneal and/or intradermal injection of emulsions containing about 100 μgs of peptide or carrier protein and Freund's adjuvant. Several booster

injections may be needed, for instance, at intervals of about two weeks, to provide a useful titer of anti-peptide antibody, which can be detected, for example, by ELISA assay using free peptide adsorbed to a solid surface. The titer of anti-peptide antibodies in serum from an immunized animal may be increased by selection of anti-peptide antibodies, for instance, by adsorption to the peptide on a solid support and elution of the selected antibodies according to methods well known in the art.

As one of skill in the art will appreciate, and discussed above, the polypeptides of the present invention including, but not limited to, polypeptides comprising an immunogenic or antigenic epitope can be fused to heterologous polypeptide sequences. For example, the polypeptides of the present invention may be fused with the constant region comprising portions of immunoglobulins (IgA, IgE, IgG, IgM), or portions of the constant region (CH1, CH2, CH3, any combination thereof including both entire domains and portions thereof) resulting in chimeric polypeptides. These fusion proteins facilitate purification, and show an increased half-life *in vivo*. This has been shown, e.g., for chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins (See, e.g., EPA 0,394,827; and Traunecker et al., 1988). Fusion proteins that have a disulfide-linked dimeric structure due to the IgG portion can also be more efficient in binding and neutralizing other molecules than monomeric polypeptides or fragments thereof alone (See, e.g., Fountoulakis et al., 1995). Nucleic acids encoding the above epitopes can also be recombined with a gene of interest as an epitope tag to aid in detection and purification of the expressed polypeptide.

Additional fusion proteins of the invention may be generated through the techniques of gene-shuffling, motif-shuffling, exon-shuffling, or codon-shuffling (collectively referred to as "DNA shuffling"). DNA shuffling may be employed to modulate the activities of polypeptides of the present invention thereby effectively generating agonists and antagonists of the polypeptides. See, for example, U.S. Patent Nos.: 5,605,793; 5,811,238; 5,834,252; 5,837,458; and Patten, P.A., et al., (1997); Harayama, S., (1998); Hansson, L.O., et al (1999); and Lorenzo, M.M. and Blasco, R., (1998). (Each of these documents are hereby incorporated by reference). In one embodiment, one or more components, motifs, sections, parts, domains, fragments, etc., of coding polynucleotides of the invention, or the polypeptides encoded thereby may be recombined with one or more components, motifs, sections, parts, domains, fragments, etc. of one or more heterologous molecules.

Antibodies

The present invention further relates to antibodies and T-cell antigen receptors (TCR) that specifically bind the polypeptides, and more specifically, the epitopes of the polypeptides of the present invention. The antibodies of the present invention include IgG (including IgG1, IgG2, IgG3, and

IgG4), IgA (including IgA1 and IgA2), IgD, IgE, or IgM, and IgY. As used herein, the term "antibody" (Ab) is meant to include whole antibodies, including single-chain whole antibodies, and antigen binding fragments thereof. In a preferred embodiment the antibodies are human antigen binding antibody fragments of the present invention include, but are not limited to, Fab, Fab' F(ab)2 and F(ab')2, Fd, single-chain Fvs (scFv), single-chain antibodies, disulfide-linked Fvs (sdFv) and fragments comprising either a V_L or V_H domain. The antibodies may be from any animal origin including birds and mammals. Preferably, the antibodies are human, murine, rabbit, goat, guinea pig, camel, horse, or chicken.

Antigen-binding antibody fragments, including single-chain antibodies, may comprise the variable region(s) alone or in combination with the entire or partial of the following: hinge region, CH1, CH2, and CH3 domains. Also included in the invention are any combinations of variable region(s) and hinge region, CH1, CH2, and CH3 domains. The present invention further includes chimeric, humanized, and human monoclonal and polyclonal antibodies, which specifically bind the polypeptides of the present invention. The present invention further includes antibodies that are anti-idiotypic to the antibodies of the present invention.

The antibodies of the present invention may be monospecific, bispecific, and trispecific or have greater multispecificity. Multispecific antibodies may be specific for different epitopes of a polypeptide of the present invention or may be specific for both a polypeptide of the present invention as well as for heterologous compositions, such as a heterologous polypeptide or solid support material. See, e.g., WO 93/17715; WO 92/08802; WO 91/00360; WO 92/05793; Tutt, A. et al. (1991); US Patents 5,573,920, 4,474,893, 5,601,819, 4,714,681, 4,925,648; Kostelny, S.A. et al. (1992).

Antibodies of the present invention may be described or specified in terms of the epitope(s) or epitope-bearing portion(s) of a polypeptide of the present invention, which are recognized or specifically bound by the antibody. In the case of proteins of the present invention secreted proteins, the antibodies may specifically bind a full-length protein encoded by a nucleic acid of the present invention, a mature protein (i.e., the protein generated by cleavage of the signal peptide) encoded by a nucleic acid of the present invention, a signal peptide encoded by a nucleic acid of the present invention, or any other polypeptide of the present invention. Therefore, the epitope(s) or epitope bearing polypeptide portion(s) may be specified as described herein, e.g., by N-terminal and C-terminal positions, by size in contiguous amino acid residues, or otherwise described herein. Therefore, the present invention includes antibodies that specifically bind specified polypeptides of the present invention, and allows for the exclusion of the same.

Antibodies of the present invention may also be described or specified in terms of their cross-reactivity. Antibodies that do not specifically bind any other analog, ortholog, or homolog of the polypeptides of the present invention are included. Antibodies that do not bind polypeptides with less

than 95%, less than 90%, less than 85%, less than 80%, less than 75%, less than 70%, less than 65%, less than 60%, less than 55%, and less than 50% identity (as calculated using methods known in the art and described herein, eg., using FASTDB and the parameters set forth herein) to a polypeptide of the present invention are also included in the present invention. Further included in the present invention are antibodies, which only bind polypeptides encoded by polynucleotides, which hybridize to a polynucleotide of the present invention under stringent hybridization conditions (as described herein). Antibodies of the present invention may also be described or specified in terms of their binding affinity. Preferred binding affinities include those with a dissociation constant or Kd value less than $5X10^{-6}M$, $10^{-6}M$, $5X10^{-7}M$, $10^{-7}M$, $5X10^{-8}M$, $10^{-8}M$, $5X10^{-9}M$, $10^{-9}M$, $5X10^{-10}M$, $10^{-10}M$, $5X10^{-11}M$, $10^{-11}M$, $5X10^{-12}M$, $10^{-12}M$, $5X10^{-13}M$, $10^{-13}M$, $5X10^{-14}M$, $10^{-14}M$, $5X10^{-15}M$, and $10^{-15}M$.

Antibodies of the present invention have uses that include, but are not limited to, methods known in the art to purify, detect, and target the polypeptides of the present invention including both *in vitro* and *in vivo* diagnostic and therapeutic methods. For example, the antibodies have use in immunoassays for qualitatively and quantitatively measuring levels of the polypeptides of the present invention in biological samples (See, e.g., Harlow et al., 1988).

The antibodies of the present invention may be used either alone or in combination with other compositions. The antibodies may further be recombinantly fused to a heterologous polypeptide at the N- or C-terminus or chemically conjugated (including covalent and non-covalent conjugations) to polypeptides or other compositions. For example, antibodies of the present invention may be recombinantly fused or conjugated to molecules useful as labels in detection assays and effector molecules such as heterologous polypeptides, drugs, or toxins. See, e.g., WO 92/08495; WO 91/14438; WO 89/12624; US Patent 5,314,995; and EP 0 396 387.

The antibodies of the present invention may be prepared by any suitable method known in the art. For example, a polypeptide of the present invention or an antigenic fragment thereof can be administered to an animal in order to induce the production of sera containing polyclonal antibodies. The term "monoclonal antibody" is not limited to antibodies produced through hybridoma technology. The term "antibody" refers to a polypeptide or group of polypeptides which are comprised of at least one binding domain, where a binding domain is formed from the folding of variable domains of an antibody molecule to form three-dimensional binding spaces with an internal surface shape and charge distribution complementary to the features of an antigenic determinant of an antigen, which allows an immunological reaction with the antigen. The term "monoclonal antibody" refers to an antibody that is derived from a single clone, including eukaryotic, prokaryotic, or phage clone, and not the method by which it is produced. Monoclonal antibodies can be prepared using a wide variety of techniques known in the art including the use of hybridoma, recombinant, and phage display technology.

Hybridoma techniques include those known in the art (See, e.g., Harlow et al. 1988); Hammerling, et al, 1981) (said references incorporated by reference in their entireties). Fab and F(ab')2 fragments may be produced, for example, from hybridoma-produced antibodies by proteolytic cleavage, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab')2 fragments).

Alternatively, antibodies of the present invention can be produced through the application of recombinant DNA technology or through synthetic chemistry using methods known in the art. For example, the antibodies of the present invention can be prepared using various phage display methods known in the art. In phage display methods, functional antibody domains are displayed on the surface of a phage particle, which carries polynucleotide sequences encoding them. Phage with a desired binding property are selected from a repertoire or combinatorial antibody library (e.g. human or murine) by selecting directly with antigen, typically antigen bound or captured to a solid surface or bead. Phage used in these methods are typically filamentous phage including fd and M13 with Fab, Fv or disulfide stabilized Fv antibody domains recombinantly fused to either the phage gene III or gene VIII protein. Examples of phage display methods that can be used to make the antibodies of the present invention include those disclosed in Brinkman U. et al. (1995); Ames, R.S. et al. (1995); Kettleborough, C.A. et al. (1994); Persic, L. et al. (1997); Burton, D.R. et al. (1994); PCT/GB91/01134; WO 90/02809; WO 91/10737; WO 92/01047; WO 92/18619; WO 93/11236; WO 95/15982; WO 95/20401; and US Patents 5,698,426, 5,223,409, 5,403,484, 5,580,717, 5,427,908, 5,750,753, 5,821,047, 5,571,698, 5,427,908, 5,516,637, 5,780,225, 5,658,727 and 5,733,743.

As described in the above references, after phage selection, the antibody coding regions from the phage can be isolated and used to generate whole antibodies, including human antibodies, or any other desired antigen binding fragment, and expressed in any desired host including mammalian cells, insect cells, plant cells, yeast, and bacteria. For example, techniques to recombinantly produce Fab, Fab' F(ab)2 and F(ab')2 fragments can also be employed using methods known in the art such as those disclosed in WO 92/22324; Mullinax, R.L. et al. (1992); and Sawai, H. et al. (1995); and Better, M. et al. (1988).

Examples of techniques which can be used to produce single-chain Fvs and antibodies include those described in U.S. Patents 4,946,778 and 5,258,498; Huston et al. (1991); Shu, L. et al. (1993); and Skerra, A. et al. (1988). For some uses, including *in vivo* use of antibodies in humans and *in vitro* detection assays, it may be preferable to use chimeric, humanized, or human antibodies. Methods for producing chimeric antibodies are known in the art. See e.g., Morrison, (1985); Oi et al., (1986); Gillies, S.D. et al. (1989); and US Patent 5,807,715. Antibodies can be humanized using a variety of techniques including CDR-grafting (EP 0 239 400; WO 91/09967; US Patent 5,530,101; and 5,585,089), veneering or resurfacing, (EP 0 592 106; EP 0 519 596; Padlan E.A., 1991; Studnicka G.M. et al., 1994;

Roguska M.A. et al., 1994), and chain shuffling (US Patent 5,565,332). Human antibodies can be made by a variety of methods known in the art including phage display methods described above. See also, US Patents 4,444,887, 4,716,111, 5,545,806, and 5,814,318; WO 98/46645; WO 98/50433; WO 98/24893; WO 96/34096; WO 96/33735; and WO 91/10741.

Further included in the present invention are antibodies recombinantly fused or chemically conjugated (including both covalently and non-covalently conjugations) to a polypeptide of the present invention. The antibodies may be specific for antigens other than polypeptides of the present invention. For example, antibodies may be used to target the polypeptides of the present invention to particular cell types, either *in vitro* or *in vivo*, by fusing or conjugating the polypeptides of the present invention to antibodies specific for particular cell surface receptors. Antibodies fused or conjugated to the polypeptides of the present invention may also be used in *in vitro* immunoassays and purification methods using methods known in the art (See e.g., Harbor et al. *supra*; WO 93/21232; EP 0 439 095; Naramura, M. et al. 1994; US Patent 5,474,981; Gillies, S.O. et al., 1992; Fell, H.P. et al., 1991).

The present invention further includes compositions comprising the polypeptides of the present invention fused or conjugated to antibody domains other than the variable regions. For example, the polypeptides of the present invention may be fused or conjugated to an antibody Fc region, or portion thereof. The antibody portion fused to a polypeptide of the present invention may comprise the hinge region, CH1 domain, CH2 domain, and CH3 domain or any combination of whole domains or portions thereof. The polypeptides of the present invention may be fused or conjugated to the above antibody portions to increase the *in vivo* half-life of the polypeptides or for use in immunoassays using methods known in the art. The polypeptides may also be fused or conjugated to the above antibody portions to form multimers. For example, Fc portions fused to the polypeptides of the present invention can form dimers through disulfide bonding between the Fc portions. Higher multimeric forms can be made by fusing the polypeptides to portions of IgA and IgM. Methods for fusing or conjugating the polypeptides of the present invention to antibody portions are known in the art. See e.g., US Patents 5,336,603, 5,622,929, 5,359,046, 5,349,053, 5,447,851, 5,112,946; EP 0 307 434, EP 0 367 166; WO 96/04388, WO 91/06570; Ashkenazi, A. et al. (1991); Zheng, X.X. et al. (1995); and Vil, H. et al. (1992).

The invention further relates to antibodies that act as agonists or antagonists of the polypeptides of the present invention. For example, the present invention includes antibodies that disrupt the receptor/ligand interactions with the polypeptides of the invention either partially or fully. Included are both receptor-specific antibodies and ligand-specific antibodies. Included are receptor-specific antibodies, which do not prevent ligand binding but prevent receptor activation. Receptor activation (i.e., signaling) may be determined by techniques described herein or otherwise known in the art. Also include are receptor-specific antibodies which both prevent ligand binding and receptor activation.

Likewise, included are neutralizing antibodies that bind the ligand and prevent binding of the ligand to the receptor, as well as antibodies that bind the ligand, thereby preventing receptor activation, but do not prevent the ligand from binding the receptor. Further included are antibodies that activate the receptor. These antibodies may act as agonists for either all or less than all of the biological activities affected by ligand-mediated receptor activation. The antibodies may be specified as agonists or antagonists for biological activities comprising specific activities disclosed herein. The above antibody agonists can be made using methods known in the art. See e.g., WO 96/40281; US Patent 5,811,097; Deng, B. et al. (1998); Chen, Z. et al. (1998); Harrop, J.A. et al. (1998); Zhu, Z. et al. (1998); Yoon, D.Y. et al. (1998); Prat, M. et al. (1998) J.; Pitard, V. et al. (1997); Liautard, J. et al. (1997); Carlson, N.G. et al. (1997) J.; Taryman, R.E. et al. (1995); Muller, Y.A. et al. (1998); Bartunek, P. et al. (1996).

As discussed above, antibodies of the polypeptides of the invention can, in turn, be utilized to generate anti-idiotypic antibodies that "mimic" polypeptides of the invention using techniques well known to those skilled in the art (See, e.g. Greenspan and Bona (1989); and Nissinoff (1991). For example, antibodies which bind to and competitively inhibit polypeptide multimerization or binding of a polypeptide of the invention to ligand can be used to generate anti-idiotypes that "mimic" the polypeptide multimerization or binding domain and, as a consequence, bind to and neutralize polypeptide or its ligand. Such neutralization anti-idiotypic antibodies can be used to bind a polypeptide of the invention or to bind its ligands/receptors, and therby block its biological activity,

The invention also concerns a purified or isolated antibody capable of specifically binding to a mutated full length or mature polypeptide of the present invention or to a fragment or variant thereof comprising an epitope of the mutated polypeptide. In another preferred embodiment, the present invention concerns an antibody capable of binding to a polypeptide comprising at least 10 consecutive amino acids of a polypeptide of the present invention and including at least one of the amino acids which can be encoded by the trait causing mutations.

Non-human animals or mammals, whether wild-type or transgenic, which express a different species of a polypeptide of the present invention than the one to which antibody binding is desired, and animals which do not express a polypeptide of the present invention (i.e. a knockout animal) are particularly useful for preparing antibodies. Gene knock out animals will recognize all or most of the exposed regions of a polypeptide of the present invention as foreign antigens, and therefore produce antibodies with a wider array of epitopes. Moreover, smaller polypeptides with only 10 to 30 amino acids may be useful in obtaining specific binding to any one of the polypeptides of the present invention. In addition, the humoral immune system of animals that produce a species of a polypeptide of the present invention that resembles the antigenic sequence will preferentially recognize the differences between the animal's native polypeptide species and the antigen sequence, and produce antibodies to

these unique sites in the antigen sequence. Such a technique will be particularly useful in obtaining antibodies that specifically bind to any one of the polypeptides of the present invention.

Antibody preparations prepared according to either protocol are useful in quantitative immunoassays which determine concentrations of antigen-bearing substances in biological samples; they are also used semi-quantitatively or qualitatively to identify the presence of antigen in a biological sample. The antibodies may also be used in therapeutic compositions for killing cells expressing the protein or reducing the levels of the protein in the body.

The antibodies of the invention may be labelled by any one of the radioactive, fluorescent or enzymatic labels known in the art.

Consequently, the invention is also directed to a method for detecting specifically the presence of a polypeptide of the present invention according to the invention in a biological sample, said method comprising the following steps:

a) obtaining a biological sample suspected of containing a polypeptide of the present invention;

b) contacting the biological sample with a polyclonal or monoclonal antibody that specifically binds a polypeptide of the present invention under conditions suitable for antigen-antibody binding; and

c) detecting the antigen-antibody complex formed.

The invention also concerns a diagnostic kit for detecting *in vitro* the presence of a polypeptide of the present invention in a biological sample, wherein said kit comprises:

a) a polyclonal or monoclonal antibody that specifically binds a polypeptide of the present invention, optionally labelled;

b) a reagent allowing the detection of the antigen-antibody complexes formed, said reagent carrying optionally a label, or being able to be recognized itself by a labelled reagent, more particularly in the case when the above-mentioned monoclonal or polyclonal antibody is not labelled by itself.

A. Monoclonal Antibody Production by Hybridoma Fusion

Monoclonal antibody to epitopes of any of the peptides identified and isolated as described can be prepared from murine hybridomas according to the classical method of Kohler, G. and Milstein, C., Nature 256:495 (1975) or derivative methods thereof. Briefly, a mouse is repetitively inoculated with a few micrograms of the selected protein or peptides derived therefrom over a period of a few weeks. The mouse is then sacrificed, and the antibody producing cells of the spleen isolated. The spleen cells are fused by means of polyethylene glycol with mouse myeloma cells, and the excess unfused cells destroyed by growth of the system on selective media comprising aminopterin (HAT media). The successfully fused cells are diluted and aliquots of the dilution placed in wells of a microtiter plate where growth of the culture is continued. Antibody-producing clones are identified by detection of

antibody in the supernatant fluid of the wells by immunoassay procedures, such as Elisa, as originally described by Engvall, E., Meth Enzymol 70:419 (1980), and derivative methods thereof. Selected positive clones can be expanded and their monoclonal antibody product harvested for use. Detailed procedures for monoclonal antibody production are described in Davis, L. et al. Basic Methods in Molecular Biology Elsevier, New York. Section 21-2.

In a preferred embodiment, said monoclonal antibody is specific for an XCRF polypeptide or polypeptide fragment comprising all or part of the globular C-terminal C1q homology domain. In preferred embodiments, said polypeptide fragment comprises, consists essentially of, or consists of, at least 6 consecutive amino acids and not more than 258 consecutive amino acids of SEQ ID NO: 2 or not more than 285 consecutive amino acids of SEQ ID NO: 4 or not more than 238 consecutive amino acids of SEQ ID NO: 6 or not more than 287 consecutive amino acids of SEQ ID NO: 8 .

In further preferred embodiment, said XCRF1 polypeptide or polypeptide fragment comprising all or part of the globular C-terminal C1q homology domain is selected from amino acids 17-258, 30-258, 39-258, 46-258, 72-258, 75-258, 78-258, 99-258, 100-258, 105-258, 110-258, 115-258, 118-258, 119-258, 120-258, 121-258, 122-258, 123-258, 124-258, 125-258, 126-258, 127-258, 128-258, 129-258 or 130-258 of SEQ ID NO: 2, where it is understood that amino acid 17 is taken to represent the N-terminal amino acid of mature XCRF1 polypeptide absent the signal peptide.

In further preferred embodiment, said XCRF3 polypeptide or polypeptide fragment comprising all or part of the globular C-terminal C1q homology domain is selected from amino acids 22-287, 123-287, 124-287, 125-287, 126-287, 127-287, 128-287, 129-287, 130-287, 131-287, 132-287, 133-287, 134-287, 135-287, 136-287, 137-287, 138-287, 139-287, 140-287, 141-287, 142-287, 143-287, 144-287, 145-287, 146-287, 147-287, 148-287, 149-287, 150-287, 151-287, 152-287 or 153-287 of SEQ ID NO: 4, where it is understood that amino acid 22 is taken to represent the N-terminal amino acid of mature XCRF3 polypeptide absent the signal peptide.

In further preferred embodiment, said XCRF4 polypeptide or polypeptide fragment comprising all or part of the globular C-terminal C1q homology domain is selected from amino acids 16-238, 97-238, 98-238, 99-238, 100-238, 101-238, 102-238, 103-238, 104-238, 105-238, 106-238, 107-238, 108-238, 109-238 or 110-238 of SEQ ID NO: 6, where it is understood that amino acid 16 is taken to represent the N-terminal amino acid of mature XCRF4 polypeptide absent the signal peptide.

In further preferred embodiment, said XCRF5 polypeptide or polypeptide fragment comprising all or part of the globular C-terminal C1q homology domain is selected from amino acids 22-287, 123-287, 124-287, 125-287, 126-287, 127-287, 128-287, 129-287, 130-287, 131-287, 132-287, 133-287, 134-287, 135-287, 136-287, 137-287, 138-287, 139-287, 140-287, 141-287, 142-287, 143-287, 144-287,

145-287, 146-287, 147-287, 148-287, 149-287, 150-287, 151-287, 152-287 or 153-287 of SEQ ID NO: 8, where it is understood that amino acid 22 is taken to represent the N-terminal amino acid of mature XCRF5 polypeptide absent the signal peptide.

B. Polyclonal Antibody Production by Immunization

Polyclonal antiserum containing antibodies to heterogenous epitopes of a single protein can be prepared by immunizing suitable animals with the expressed protein or peptides derived therefrom described above, which can be unmodified or modified to enhance immunogenicity. Effective polyclonal antibody production is affected by many factors related both to the antigen and the host species. For example, small molecules tend to be less immunogenic than others and may require the use of carriers and adjuvant. Also, host animals vary in response to site of inoculations and dose, with both inadequate or excessive doses of antigen resulting in low titer antisera. Small doses (ng level) of antigen administered at multiple intradermal sites appears to be most reliable. An effective immunization protocol for rabbits can be found in Vaitukaitis, J. et al. J. Clin. Endocrinol. Metab. 33:988-991 (1971).

Booster injections can be given at regular intervals, and antiserum harvested when antibody titer thereof, as determined semi-quantitatively, for example, by double immunodiffusion in agar against known concentrations of the antigen, begins to fall. See, for example, Ouchterlony, O. et al., Chap. 19 in: Handbook of Experimental Immunology D. Wier (ed) Blackwell (1973). Plateau concentration of antibody is usually in the range of 0.1 to 0.2 mg/ml of serum (about 12 M). Affinity of the antisera for the antigen is determined by preparing competitive binding curves, as described, for example, by Fisher, D., Chap. 42 in: Manual of Clinical Immunology, 2d Ed. (Rose and Friedman, Eds.) Amer. Soc. For Microbiol., Washington, D.C. (1980).

In a preferred embodiment, said polyclonal antibody is specific for an XCRF1 polypeptide or polypeptide fragment comprising all or part of the globular C-terminal C1q homology domain. In preferred embodiments, said polypeptide fragment comprises, consists essentially of, or consists of, at least 6 consecutive amino acids and not more than 258 consecutive amino acids of SEQ ID NO: 2. In further preferred embodiment, said XCRF1 polypeptide or polypeptide fragment comprising all or part of the globular C-terminal C1q homology domain is selected from amino acids 17-258, 30-258, 39-258, 46-258, 72-258, 75-258, 78-258, 99-258, 100-258, 105-258, 110-258, 115-258, 118-258, 119-258, 120-258, 121-258, 122-258, 123-258, 124-258, 125-258, 126-258, 127-258, 128-258, 129-258 or 130-258 of SEQ ID NO: 2, where it is understood that amino acid 17 is taken to represent the N-terminal amino acid of mature XCRF1 polypeptide absent the signal peptide.

In a preferred embodiment, said polyclonal antibody is specific for a XCRF3 polypeptide or polypeptide fragment comprising all or part of the globular C-terminal C1q homology domain. In

preferred embodiments, said polypeptide fragment comprises, consists essentially of, or consists of, at least 6 consecutive amino acids and not more than 287 consecutive amino acids of SEQ ID NO: 4. In further preferred embodiment, said XCRF3 polypeptide or polypeptide fragment comprising all or part of the globular C-terminal C1q homology domain is selected from amino acids 22-287, 123-287, 124-287, 125-287, 126-287, 127-287, 128-287, 129-287, 130-287, 131-287, 132-287, 133-287, 134-287, 135-287, 136-287, 137-287, 138-287, 139-287, 140-287, 141-287, 142-287, 143-287, 144-287, 145-287, 146-287, 147-287, 148-287, 149-287, 150-287, 151-287, 152-287 or 153-287 of SEQ ID NO: 4, where it is understood that amino acid 22 is taken to represent the N-terminal amino acid of mature XCRF3 polypeptide absent the signal peptide.

In a preferred embodiment, said polyclonal antibody is specific for an XCRF4 polypeptide or polypeptide fragment comprising all or part of the globular C-terminal C1q homology domain. In preferred embodiments, said polypeptide fragment comprises, consists essentially of, or consists of, at least 6 consecutive amino acids and not more than 258 consecutive amino acids of SEQ ID NO: 6. In further preferred embodiment, said XCRF4 polypeptide or polypeptide fragment comprising all or part of the globular C-terminal C1q homology domain is selected from amino acids 16-238, 97-238, 98-238, 99-238, 100-238, 101-238, 102-238, 103-238, 104-238, 105-238, 106-238, 107-238, 108-238, 109-238 or 110-238 of SEQ ID NO: 6, where it is understood that amino acid 16 is taken to represent the N-terminal amino acid of mature XCRF4 polypeptide absent the signal peptide.

In a preferred embodiment, said polyclonal antibody is specific for an XCRF5 polypeptide or polypeptide fragment comprising all or part of the globular C-terminal C1q homology domain. In preferred embodiments, said polypeptide fragment comprises, consists essentially of, or consists of, at least 6 consecutive amino acids and not more than 287 consecutive amino acids of SEQ ID NO: 8. In further preferred embodiment, said XCRF5 polypeptide or polypeptide fragment comprising all or part of the globular C-terminal C1q homology domain is selected from amino acids 22-287, 123-287, 124-287, 125-287, 126-287, 127-287, 128-287, 129-287, 130-287, 131-287, 132-287, 133-287, 134-287, 135-287, 136-287, 137-287, 138-287, 139-287, 140-287, 141-287, 142-287, 143-287, 144-287, 145-287, 146-287, 147-287, 148-287, 149-287, 150-287, 151-287, 152-287 or 153-287 of SEQ ID NO: 8, where it is understood that amino acid 22 is taken to represent the N-terminal amino acid of mature XCRF5 polypeptide absent the signal peptide.

Antibody preparations prepared according to either protocol are useful in quantitative immunoassays which determine concentrations of antigen-bearing substances in biological samples; they are also used semi-quantitatively or qualitatively to identify the presence of antigen in a biological sample. The antibodies may also be used in therapeutic compositions for killing cells expressing the protein or reducing the levels of the protein in the body.

XIII. Identifying One or More Cell Types Expressing a Cell Surface Receptor for XCRF Polypeptide

The invention features methods of identifying one or more cell types expressing a cell surface receptor for XCRF polypeptide comprised of contacting said cell type with labelled XCRF polypeptide and measuring the amount of said polypeptide bound. Preferably said XCRF polypeptide comprises all or part of the globular C-terminal C1q homology domain and has activity selected from the group consisting of lipid partitioning, lipid metabolism, and insulin-like activities. Also preferably said XCRF polypeptide comprises all or part of the globular C-terminal C1q homology domain and has activity selected from the group consisting of prevention of weight gain, weight reduction, and maintenance of weight loss.

In preferred embodiment, said XCRF1 polypeptide comprising all or part of the globular C-terminal C1q homology domain and having activity selected from the group consisting of lipid partitioning, lipid metabolism, and insulin-like activities comprises, consists essentially of, or consists of, at least 6 consecutive amino acids and not more than 258 consecutive amino acids of SEQ ID NO: 2. In further preferred embodiment, said XCRF1 polypeptide or polypeptide fragment comprising all or part of the globular C-terminal C1q homology domain and having activity selected from the group consisting of lipid partitioning, lipid metabolism, and insulin-like activities is selected from amino acids 17-258, 30-258, 39-258, 46-258, 72-258, 75-258, 78-258, 99-258, 100-258, 105-258, 110-258, 115-258, 118-258, 119-258, 120-258, 121-258, 122-258, 123-258, 124-258, 125-258- 126-258- 127-258, 128-258, 129-258 or 130-258 of SEQ ID NO: 2, where it is understood that amino acid 17 is taken to represent the N-terminal amino acid of mature XCRF polypeptide absent the signal peptide. In other further preferred embodiment, said XCRF1 polypeptide or polypeptide fragment comprising all or part of the globular C-terminal C1q homology domain and having activity selected from the group consisting of prevention of weight gain, weight reduction, and maintenance of weight loss is selected from amino acids 17-258, 30-258, 39-258, 46-258, 72-258, 75-258, 78-258, 99-258, 100-258, 105-258, 110-258, 115-258, 118-258, 119-258, 120-258, 121-258, 122-258, 123-258, 124-258, 125-258- 126-258- 127-258, 128-258, 129-258 or 130-258 of SEQ ID NO: 2, where it is understood that amino acid 17 is taken to represent the N-terminal amino acid of mature XCRF1 polypeptide absent the signal peptide.

In preferred embodiment, said XCRF3 polypeptide comprising all or part of the globular C-terminal C1q homology domain and having activity selected from the group consisting of lipid partitioning, lipid metabolism, and insulin-like activities comprises, consists essentially of, or consists of, at least 6 consecutive amino acids and not more than 287 consecutive amino acids of SEQ ID NO: 4. In further preferred embodiment, said XCRF3 polypeptide or polypeptide fragment comprising all or part of the globular C-terminal C1q homology domain and having activity selected from the group consisting of lipid

partitioning, lipid metabolism, and insulin-like activities is selected from amino acids 22-287, 123-287, 124-287, 125-287, 126-287, 127-287, 128-287, 129-287, 130-287, 131-287, 132-287, 133-287, 134-287, 135-287, 136-287, 137-287, 138-287, 139-287, 140-287, 141-287, 142-287, 143-287, 144-287, 145-287, 146-287, 147-287, 148-287, 149-287, 150-287, 151-287, 152-287 or 153-287 of SEQ ID NO: 4, where it is understood that amino acid 22 is taken to represent the N-terminal amino acid of mature XCRF3 polypeptide absent the signal peptide. In other further preferred embodiment, said XCRF3 polypeptide or polypeptide fragment comprising all or part of the globular C-terminal C1q homology domain and having activity selected from the group consisting of prevention of weight gain, weight reduction, and maintenance of weight loss is selected from amino acids 22-287, 123-287, 124-287, 125-287, 126-287, 127-287, 128-287, 129-287, 130-287, 131-287, 132-287, 133-287, 134-287, 135-287, 136-287, 137-287, 138-287, 139-287, 140-287, 141-287, 142-287, 143-287, 144-287, 145-287, 146-287, 147-287, 148-287, 149-287, 150-287, 151-287, 152-287 or 153-287 of SEQ ID NO: 4, where it is understood that amino acid 22 is taken to represent the N-terminal amino acid of mature XCRF3 polypeptide absent the signal peptide.

In preferred embodiment, said XCRF4 polypeptide comprising all or part of the globular C-terminal C1q homology domain and having activity selected from the group consisting of lipid partitioning, lipid metabolism, and insulin-like activities comprises, consists essentially of, or consists of, at least 6 consecutive amino acids and not more than 238 consecutive amino acids of SEQ ID NO: 6. In further preferred embodiment, said XCRF4 polypeptide or polypeptide fragment comprising all or part of the globular C-terminal C1q homology domain and having activity selected from the group consisting of lipid partitioning, lipid metabolism, and insulin-like activities is selected from amino acids 16-238, 97-238, 98-238, 99-238, 100-238, 101-238, 102-238, 103-238, 104-238, 105-238, 106-238, 107-238, 108-238, 109-238 or 110-238 of SEQ ID NO: 6, where it is understood that amino acid 16 is taken to represent the N-terminal amino acid of mature XCRF4 polypeptide absent the signal peptide. In other further preferred embodiment, said XCRF4 polypeptide or polypeptide fragment comprising all or part of the globular C-terminal C1q homology domain and having activity selected from the group consisting of prevention of weight gain, weight reduction, and maintenance of weight loss is selected from amino acids 16-238, 97-238, 98-238, 99-238, 100-238, 101-238, 102-238, 103-238, 104-238, 105-238, 106-238, 107-238, 108-238, 109-238 or 110-238 of SEQ ID NO: 6, where it is understood that amino acid 16 is taken to represent the N-terminal amino acid of mature XCRF4 polypeptide absent the signal peptide.

In preferred embodiment, said XCRF5 polypeptide comprising all or part of the globular C-terminal C1q homology domain and having activity selected from the group consisting of lipid partitioning, lipid metabolism, and insulin-like activities comprises, consists essentially of, or consists of, at least 6 consecutive amino acids and not more than 287 consecutive amino acids of SEQ ID NO: 8. In further preferred embodiment, said XCRF5 polypeptide or polypeptide fragment comprising all or part of the

globular C-terminal C1q homology domain and having activity selected from the group consisting of lipid partitioning, lipid metabolism, and insulin-like activities is selected from amino acids 22-287, 123-287, 124-287, 125-287, 126-287, 127-287, 128-287, 129-287, 130-287, 131-287, 132-287, 133-287, 134-287, 135-287, 136-287, 137-287, 138-287, 139-287, 140-287, 141-287, 142-287, 143-287, 144-287, 145-287, 146-287, 147-287, 148-287, 149-287, 150-287, 151-287, 152-287 or 153-287 of SEQ ID NO: 8, where it is understood that amino acid 22 is taken to represent the N-terminal amino acid of mature XCRF5 polypeptide absent the signal peptide. In other further preferred embodiment, said XCRF5 polypeptide or polypeptide fragment comprising all or part of the globular C-terminal C1q homology domain and having activity selected from the group consisting of prevention of weight gain, weight reduction, and maintenance of weight loss is selected from amino acids 22-287, 123-287, 124-287, 125-287, 126-287, 127-287, 128-287, 129-287, 130-287, 131-287, 132-287, 133-287, 134-287, 135-287, 136-287, 137-287, 138-287, 139-287, 140-287, 141-287, 142-287, 143-287, 144-287, 145-287, 146-287, 147-287, 148-287, 149-287, 150-287, 151-287, 152-287 or 153-287 of SEQ ID NO: 8, where it is understood that amino acid 22 is taken to represent the N-terminal amino acid of mature XCRF5 polypeptide absent the signal peptide.

Preferably said labelled XCRF polypeptide is selected from but not restricted to fluorescein-coupled XCRF or biotin-coupled XCRF. Bound fluorescein-coupled XCRF is detected directly by FACS. Bound biotin-coupled XCRF is detected by FACS after secondary binding of phycoerythrin-coupled streptavidin or by radioassay after secondary binding of ^{125}I-streptavidin. Alternatively, XCRF polypeptide is tagged with an antibody epitope at the N- or C-terminus as described *supra* with regard to polynucleotides encoding polypeptides of the invention that are fused in frame to the coding sequences for additional heterologous amino acid sequences. Binding of said epitope-tagged XCRF polypeptide is detected with antibody specific for the epitope.

XIV. <u>Cloning cDNA Encoding Cell Surface Receptor for XCRF Polypeptide</u>

The invention features methods of using XCRF polypeptide to clone cDNA encoding a cell surface receptor for said XCRF polypeptide. Preferably said XCRF polypeptide comprises all or part of the globular C-terminal C1q homology domain and has activity selected from the group consisting of lipid partitioning, lipid metabolism, and insulin-like activities. Also preferably said XCRF polypeptide comprises all or part of the globular C-terminal C1q homology domain and has activity selected from the group consisting of prevention of weight gain, weight reduction, and maintenance of weight loss.

In preferred embodiment, said XCRF1 polypeptide comprising all or part of the globular C-terminal C1q homology domain and having activity selected from the group consisting of lipid partitioning, lipid metabolism, and insulin-like activities comprises, consists essentially of, or consists of, at least 6 consecutive amino acids and not more than 258 consecutive amino acids of SEQ ID NO: 2. In

further preferred embodiment, said XCRF1 polypeptide or polypeptide fragment comprising all or part of the globular C-terminal C1q homology domain and having activity selected from the group consisting of lipid partitioning, lipid metabolism, and insulin-like activities is selected from amino acids 17-258, 30-258, 39-258, 46-258, 72-258, 75-258, 78-258, 99-258, 100-258, 105-258, 110-258, 115-258, 118-258, 119-258, 120-258, 121-258, 122-258, 123-258, 124-258, 125-258, 126-258, 127-258, 128-258, 129-258 or 130-258 of SEQ ID NO: 2, where it is understood that amino acid 17 is taken to represent the N-terminal amino acid of mature XCRF1 polypeptide absent the signal peptide. In other further preferred embodiment, said XCRF1 polypeptide or polypeptide fragment comprising all or part of the globular C-terminal C1q homology domain and having activity selected from the group consisting of prevention of weight gain, weight reduction, and maintenance of weight loss is selected from amino acids 17-258, 30-258, 39-258, 46-258, 72-258, 75-258, 78-258, 99-258, 100-258, 105-258, 110-258, 115-258, 118-258, 119-258, 120-258, 121-258, 122-258, 123-258, 124-258, 125-258- 126-258- 127-258, 128-258, 129-258 or 130-258 of SEQ ID NO: 2, where it is understood that amino acid 17 is taken to represent the N-terminal amino acid of mature XCRF1 polypeptide absent the signal peptide.

In preferred embodiment, said XCRF3 polypeptide comprising all or part of the globular C-terminal C1q homology domain and having activity selected from the group consisting of lipid partitioning, lipid metabolism, and insulin-like activities comprises, consists essentially of, or consists of, at least 6 consecutive amino acids and not more than 287 consecutive amino acids of SEQ ID NO: 4. In further preferred embodiment, said XCRF3 polypeptide or polypeptide fragment comprising all or part of the globular C-terminal C1q homology domain and having activity selected from the group consisting of lipid partitioning, lipid metabolism, and insulin-like activities is selected from amino acids 22-287, 123-287, 124-287, 125-287, 126-287, 127-287, 128-287, 129-287, 130-287, 131-287, 132-287, 133-287, 134-287, 135-287, 136-287, 137-287, 138-287, 139-287, 140-287, 141-287, 142-287, 143-287, 144-287, 145-287, 146-287, 147-287, 148-287, 149-287, 150-287, 151-287, 152-287 or 153-287 of SEQ ID NO: 4, where it is understood that amino acid 22 is taken to represent the N-terminal amino acid of mature XCRF3 polypeptide absent the signal peptide.

In preferred embodiment, said XCRF4 polypeptide comprising all or part of the globular C-terminal C1q homology domain and having activity selected from the group consisting of lipid partitioning, lipid metabolism, and insulin-like activities comprises, consists essentially of, or consists of, at least 6 consecutive amino acids and not more than 238 consecutive amino acids of SEQ ID NO: 6. In further preferred embodiment, said XCRF4 polypeptide or polypeptide fragment comprising all or part of the globular C-terminal C1q homology domain and having activity selected from the group consisting of lipid partitioning, lipid metabolism, and insulin-like activities is selected from amino acids 16-238, 97-238, 98-238, 99-238, 100-238, 101-238, 102-238, 103-238, 104-238, 105-238, 106-238, 107-238, 108-238, 109-238

or 110-238 of SEQ ID NO: 6, where it is understood that amino acid 16 is taken to represent the N-terminal amino acid of mature XCRF4 polypeptide absent the signal peptide.

In preferred embodiment, said XCRF5 polypeptide comprising all or part of the globular C-terminal C1q homology domain and having activity selected from the group consisting of lipid partitioning, lipid metabolism, and insulin-like activities comprises, consists essentially of, or consists of, at least 6 consecutive amino acids and not more than 287 consecutive amino acids of SEQ ID NO: 8. In further preferred embodiment, said XCRF5 polypeptide or polypeptide fragment comprising all or part of the globular C-terminal C1q homology domain and having activity selected from the group consisting of lipid partitioning, lipid metabolism, and insulin-like activities is selected from amino acids 22-287, 123-287, 124-287, 125-287, 126-287, 127-287, 128-287, 129-287, 130-287, 131-287, 132-287, 133-287, 134-287, 135-287, 136-287, 137-287, 138-287, 139-287, 140-287, 141-287, 142-287, 143-287, 144-287, 145-287, 146-287, 147-287, 148-287, 149-287, 150-287, 151-287, 152-287 or 153-287 of SEQ ID NO: 8, where it is understood that amino acid 22 is taken to represent the N-terminal amino acid of mature XCRF5 polypeptide absent the signal peptide.

In a preferred embodiment, said method of cloning a cell surface receptor for XCRF polypeptide comprises: isolating mRNA from a cell type expressing said cell surface receptor for XCRF polypeptide; converting said mRNA to cDNA; ligating said cDNA into a eukaryotic expression vector containing the origin for SV40 replication; transiently transfecting pools of said ligated cDNA into COS cells using dextran sulfate; culturing the transfected COS cells for about 48 h; detecting cell surface expression of said receptor for XCRF polypeptide by contacting said transfected COS cells with biotinylated or epitope-tagged XCRF polypeptide; contacting said biotinylated or epitope-tagged XCRF polypeptide bound to said transfected COS cells directly (biotinylated said polypeptide) or indirectly (epitope-tagged said polypeptide) with ^{125}I-streptavidin; identifying said transfected COS cells labelled with ^{125}I-streptavidin; recovering cDNA from said labelled COS cells; and repeating said transient transfection with smaller pools of said recovered cDNA until transfection with a single clone of cDNA leads to cell surface expression of said receptor for XCRF polypeptide. Said method of cloning cDNA encoding a cell surface receptor for XCRF polypeptide by transient transfection of COS cells is well known to those skilled in the art.

Other characteristics and advantages of the invention are described in the Examples. These are meant to be exemplary only, and not to limit the invention in any way. Throughout this application, various publications, patents and published patent applications are cited. The disclosures of these publications, patents and published patent specifications referenced in this application are hereby incorporated by reference into the present disclosure.

EXAMPLES

The following Examples are provided for illustrative purposes and not as a means of limitation. One of ordinary skill in the art would be able to design equivalent assays and methods based on the disclosure herein all of which form part of the instant invention.

EXAMPLE 1: Northern Analysis of XCRF mRNA

Analysis of XCRF expression in different human tissues (adult and fetal) and cell lines, as well as mouse embryos in different stages of development, is accomplished by using poly A^+ RNA blots purchased from Clontech (e.g. #7780-1, 7757-1, 7756-1, 7768-1and 7763-1). Labeling of RNA probes is performed using the RNA Strip-EZ kit from Ambion as per manufacture's instructions. Hybridization of RNA probes to RNA blots is performed Ultrahyb hybridization solution (Ambion). Briefly, blots are prehybridized for 30 min at 58^{0}C (low-strigency) or 65^{0}C (high stringency). After adding the labelled probe (2x10^6 cpm/ml), blots are hybridized overnight (14-24 hrs), and washed 2 x 20 min at 50^{0}C with 2x SSC/0.1% SDS (low stringency), 2 x 20 min at 58^{0}C with 1x SSC/0.1%SDS (medium stringency) and 2 x 20 min at 65^{0}C with 1x SSC/0.1%SDS (high stringency). After washings are completed blots are exposed on the phosphoimager (Molecular Dynamics) for 1-3 days.

EXAMPLE 2: *In Vitro* Tests of Metabolic-related Activity

The activity of various preparations and various sequence variants of XCRF polypeptides are assessed using various *in vitro* assays including those provided below. These assays are also exemplary of those that can be used to develop XCRF polypeptide antagonists and agonists. To do that, the effect of XCRF polypeptides in the above assays, e.g. on leptin and/or LSR activity, in the presence of the candidate molecules would be compared with the effect of XCRF polypeptides in the assays in the absence of the candidate molecules. Since XCRF polypeptides reduce body weight in mice on a high-cafeteria diet (Example 5), these assays also serve to identify candidate treatments for reducing (or increasing) body weight.

Liver Cell Line:

Tests of efficacy of XCRF polypeptides on LSR can be performed using liver cell lines, including for example, PLC, HepG2, Hep3B (human), Hepa 1-6, BPRCL (mouse), or MCA-RH777, MCA-RH8994 (rat).

BPRCL mouse liver cells (ATCC Repository) are plated at a density of 300,000 cells/well in 6-well plates (day 0) in DMEM (high glucose) containing glutamine and penicillin-streptomycin (Bihain & Yen, 1992). Media is changed on day 2. On day 3, the confluent monolayers are washed once with

phosphate-buffered saline (PBS, pH 7.4) (2 mL/well). Cells are incubated at 37°C for 30 min with increasing concentrations of recombinant XCRF polypeptide or XCRF polypeptide fragment comprising the globular C-terminal C1q homology domain in DMEM containing 0.2% (w/v) BSA, 5 mM Hepes, 2 mM $CaCl_2$, 3.7 g/L sodium bicarbonate, pH 7.5. Incubations are continued for 3 h at 37°C after addition of 10 ng/mL ^{125}I-mouse leptin (specific activity, 22100 cpm/ng). Monolayers are washed 2 times consecutively with PBS containing 0.2% BSA, followed by 1 wash with PBS/BSA, and then 2 times consecutively with PBS. Cells are lysed with 0.1 N NaOH containing 0.24 mM EDTA. Lysates are collected into tubes, and counted in a gamma-counter.

Blood Brain Barrier Model:

The effect of XCRF polypeptides on leptin transport in the brain can be determined using brain-derived cells. One method that is envisioned is to use the blood/brain barrier model described by Dehouck, *et al* (J Neurochem 54:1798-801, 1990; hereby incorporated herein by reference in its entirety including any figures, tables, or drawings) that uses a co-culture of brain capillary endothelial cells and astrocytes to test the effects of XCRF polypeptides on leptin (or other molecules) transport via LSR or other receptors.

This assay would be an indicator of the potential effect of XCRF polypeptides on leptin transport to the brain and could be used to screen XCRF polypeptide variants for their ability to modulate leptin transport through LSR or other receptors in the brain. In addition, putative agonists and antagonists of the effect of XCRF polypeptides on leptin transport through LSR or other receptors could also be screened using this assay. Increased transport of leptin across the blood/brain barrier would presumably increase its action as a satiety factor.

FACS Analysis of LSR Expression

The effect of XCRF polypeptides on LSR can also be determined by measuring the level of LSR expression at the cell surface by flow surface cytometry, using anti-LSR antibodies and fluorescent secondary antibodies. Flow cytometry is a laser-based technology that is used to measure characteristics of biological particles. The underlying principle of flow cytometry is that light is scattered and fluorescence is emitted as light from the excitation source strikes the moving particles.

This is a high throughput assay that could be easily adapted to screen XCRF polypeptides and variants as well as putative agonists or antagonists of XCRF polypeptides. Two assays are provided below. The antibody, cell-line and XCRF polypeptide analogs would vary depending on the experiment, but a human cell-line, human anti-LSR antibody and XCRF polypeptide fragment comprising the globular C-terminal C1q homology domain could be used to screen for variants, agonists, and antagonists to be used to treat humans.

Assay 1:

Cells are pretreated with either intact XCRF polypeptide or XCRF polypeptide fragment comprising the globular C-terminal C1q homology domain (or untreated) before harvesting and analysis by FACS. Cells are harvested using non-enzymatic dissociation solution (Sigma), and then are incubated for 1 h at 4°C with a 1:200 dilution of anti-LSR 81B or an irrelevant anti-serum in PBS containing 1% (w/v) BSA. After washing twice with the same buffer, goat anti-rabbit FITC-conjugated antibody (Rockland, Gilbertsville, PA) is added to the cells, followed by a further incubation for 30 min at 4 °C. After washing, the cells are fixed in 2% formalin. Flow cytometry analysis is done on a FACSCalibur cytometer (Becton-Dickinson, Franklin Lakes, NJ).

Assay 2:

Cells are cultured in T175 flasks according to manufacturer's instructions for 48 hours prior to analysis.

Cells are washed once with FACS buffer (1x PBS/2% FBS, filter sterilized), and manually scraped from the flask in 10 mLs of FACS buffer. The cell suspension is transferred to a 15 mL conical tube and centrifuged at 1200 rpm, 4°C for 5 minutes. Supernatant is discarded and cells are resuspended in 10 mL FACS buffer chilled to 4°C. A cell count is performed and the cell density adjusted with FACS buffer to a concentration of 1×10^6 cells/ mL. One milliliter of cell suspension was added to each well of a 48 well plate for analysis. Cells are centrifuged at 1200 rpm for 5 minutes at 4°C. Plates are checked to ensure that cells are pelleted, the supernatant is removed and cells resuspended by running plate over a vortex mixer. One milliliter of FACS buffer is added to each well, followed by centrifugation at 1200 rpm for 5 minutes at 4°C. This described cell washing was performed a total of 3 times.

Primary antibody, titered in screening experiments to determine proper working dilutions (for example 1:25, 1:50, 1:100, 1:200, 1:400, 1:500, 1:800, 1:1000, 1:2000, 1:4000, 1:5000, or 1:10000), is added to cells in a total volume of 50 µL FACS buffer. Plates are incubated for 1h at 4°C protected from light. Following incubation, cells are washed 3 times as directed above. Appropriate secondary antibody, titered in screening experiments to determine proper working dilutions (for example 1:25, 1:50, 1:100, 1:200, 1:400, 1:500, 1:800, 1:1000, 1:2000, 1:4000, 1:5000, or 1:10000), is added to cells in a total volume of 50 µL FACS buffer. Plates are incubated for 1h at 4°C protected from light. Following incubation, cells are washed 3 times as directed above. Upon final wash, cells are resuspended in 500 µL FACS buffer and transferred to a FACS acquisition tube. Samples are placed on ice protected from light and analyzed within 1 hour.

Cellular Binding and Uptake of XCRF Polypeptides as Detected by Fluorescence Microscopy

Fluorecein isothiocyanate (FITC) conjugation of XCRF polypeptides: Purified XCRF proteins at 1 mg/mL concentration are labelled with FITC using Sigma's FluoroTag FITC conjugation kit (Stock No. FITC-1). Protocol outlined in the Sigma Handbook for small-scale conjugation is followed for XCRF protein labeling.

Cell Culture: C2C12 mouse skeletal muscle cells (ATCC, Manassas, VA CRL-1772) and Hepa-1-6 mouse hepatocytes (ATCC, Manassas, VA CRL-1830) are seeded into 6 well plates at a cell density of $2x10^5$ cells per well. C2C12 and Hepa-1-6 cells are cultured according to repository's instructions for 24-48 hours prior to analysis. Assay is performed when cells were 80% confluent.

FITC labelled XCRF protein cellular binding and uptake using microscopy: C2C12 and Hepa 1-6 cells are incubated in the presence/absence of antibody directed against human LSR (81B: N-terminal sequence of human LSR; does not cross react with mouse LSR and 93A: c-terminal sequence, cross reacts with mouse LSR) or an antiserum directed against gC1qr (953) for 1 hour at 37°C, 5% CO2. LSR antibodies are added to the media at a concentration of 2 µg/mL. The anti-gC1qr antiserum is added to the media at a volume of 2.5 µL undiluted serum (high concentration) or 1:100 dilution (low concentration). Following incubation with specified antibody, FITC-XCRF polypeptide (50 nM/mL) is added to each cell culture well. Cells are again incubated for 1 hour at 37°C, 5% CO2. Cells are washed 2x with PBS, cells are scraped from well into 1 mL of PBS. Cell suspension is transferred to an eppendorf tube and centrifuged at 1000 rpm for 2 minutes. Supernatant is removed and cells resuspended in 200 µL of PBS. Binding and uptake of FITC-XCRF polypeptide is analyzed by fluorescence microscopy under 40X magnification.

This assay may be useful for identifying agents that facilitate or prevent the uptake and/or binding of XCRF polypeptides to cells.

Effect on LSR as a Lipoprotein Receptor

The effect of XCRF protein on the lipoprotein binding, internalizing and degrading activity of LSR can also be tested. Measurement of LSR as lipoprotein receptor is described in Bihain & Yen, ((1992) Biochemistry 31:4628-36; hereby incorporated herein in its entirety including any drawings, tables, or figures). The effect of XCRF protein on the lipoprotein binding, internalizing and degrading activity of LSR (or other receptors) can be compared with that of intact XCRF protein, with untreated cells as an additional control. This assay can also be used to screen for active and inhibitory variants of XCRF protein, as well as agonists and antagonists of metabolic-related activity.

Human liver PLC cells (ATCC Repository) are plated at a density of 300,000 cells/well in 6-well plates (day 0) in DMEM (high glucose) containing glutamine and penicillin-streptomycin (Bihain & Yen, 1992). Media is changed on day 2. On day 3, the confluent monolayers are washed once with

phosphate-buffered saline (PBS, pH 7.4) (2 mL/well). Cells are incubated at 37°C for 30 min with 10 ng/mL human recombinant leptin in DMEM containing 0.2% (w/v) BSA, 5 mM Hepes, 2 mM $CaCl_2$, 3.7 g/L sodium bicarbonate, pH 7.5, followed by another 30 min incubation at 37°C with increasing concentrations of XCRF polypeptide. Incubations are continued for 2 h at 37°C after addition of 0.8 mM oleate and 20 µg/mL ^{125}I-LDL. Monolayers are washed 2 times consecutively with PBS containing 0.2% BSA, followed by 1 wash with PBS/BSA, and then 2 times consecutively with PBS. The amounts of oleate-induced binding, uptake and degradation of ^{125}I-LDL are measured as previously described (Bihain & Yen, 1992, *supra*). Results are shown as the mean of triplicate determinations.

XCRF protein leads to an increased activity of LSR as a lipoprotein receptor. The oleate-induced binding and uptake of LDL would be more affected by XCRF protein as compared to the degradation. This increased LSR activity would potentially result in an enhanced clearance of triglyceride-rich lipoproteins during the postprandial state. Thus, more dietary fat would be removed through the liver, rather than being deposited in the adipose tissue.

This assay could be used to determine the efficiency of a compound (or agonists or antagonists) to increase or decrease LSR activity (or lipoprotein uptake, binding and degradation through other receptors), and thus affect the rate of clearance of triglyceride-rich lipoproteins.

Effect on Muscle Differentiation

C2C12 cells (murine skeletal muscle cell line; ATCC CRL 1772, Rockville, MD) are seeded sparsely (about 15-20%) in complete DMEM (w/glutamine, pen/strep, etc) + 10% FCS. Two days later they become 80-90% confluent. At this time, the media is changed to DMEM+2% horse serum to allow differentiation. The media is changed daily. Abundant myotube formation occurs after 3-4 days of being in 2% horse serum, although the exact time course of C2C12 differentiation depends on how long they have been passaged and how they have been maintained, among other things.

To test the effect of the presence of XCRF protein on muscle differentiation, XCRF polypeptide or polypeptide fragment comprising the globular C-terminal C1q homology domain (1 to 2.5 µg/mL) is added the day after seeding when the cells are still in DMEM w/ 10% FCS. Two days after plating the cells (one day after said XCRF polypeptide or polypeptide fragment was first added), at about 80-90% confluency, the media is changed to DMEM+2% horse serum plus said XCRF polypeptide or polypeptide fragment.

Effect on Muscle Cell Fatty Acid Oxidation

C2C12 cells are differentiated in the presence or absence of 2 µg/mL XCRF protein for 4 days. On day 4, oleate oxidation rates are determined by measuring conversion of 1-^{14}C-oleate (0.2 mM) to

$^{14}CO_2$ for 90 min. This experiment can be used to screen for active polypeptides and peptides as well as agonists and antagonists or activators and inhibitors of XCRF polypeptides.

The effect of XCRF polypeptide or polypeptide fragment comprising the globular C-terminal C1q homology domain on the rate of oleate oxidation can be compared in differentiated C2C12 cells (murine skeletal muscle cells; ATCC, Manassas, VA CRL-1772) and in a hepatocyte cell line (Hepa1-6; ATCC, Manassas, VA CRL-1830). Cultured cells are maintained according to manufacturer's instructions. The oleate oxidation assay is performed as previously described (Muoio et al (1999) Biochem J 338;783-791). Briefly, nearly confluent myocytes are kept in low serum differentiation media (DMEM, 2.5% Horse serum) for 4 days, at which time formation of myotubes became maximal. Hepatocytes are kept in the same DMEM medium supplemented with 10% FCS for 2 days. One hour prior to the experiment the media is removed and 1 mL of preincubation media (MEM, 2.5% Horse serum, 3 mM glucose, 4 mM Glutamine, 25 mM Hepes, 1% FFA free BSA, 0.25 mM Oleate, 5 µg/mL gentamycin) is added. At the start of the oxidation experiment ^{14}C-Oleic acid (1µCi/mL, American Radiolabelled Chemical Inc., St. Louis, MO) is added and cells are incubated for 90 min at 37°C in the absence/presence of 2.5 µg/mL XCRF polypeptide or polypeptide fragment. After the incubation period 0.75 mL of the media is removed and assayed for ^{14}C-oxidation products as described below for the muscle FFA oxidation experiment.

Triglyceride and Protein Analysis following Oleate Oxidation in cultured cells

Following transfer of media for oleate oxidation assay, cells are placed on ice. To determine triglyceride and protein content, cells are washed with 1 mL of 1x PBS to remove residual media. To each well 300 µL of cell dissociation solution (Sigma) is added and incubated at 37°C for 10 min. Plates are tapped to loosen cells, and 0.5 mL of 1x PBS was added. The cell suspension is transferred to an eppendorf tube, each well is rinsed with an additional 0.5 mL of 1x PBS, and is transferred to appropriate eppendorf tube. Samples are centrifuged at 1000 rpm for 10 minutes at room temperature. Supernatant is discarded and 750 µL of 1x PBS/2% chaps is added to cell pellet. Cell suspension is vortexed and placed on ice for 1 hour. Samples are then centrifuged at 13000 rpm for 20 min at 4°C. Supernatants are transferred to new tube and frozen at –20°C until analyzed. Quantitative measure of triglyceride level in each sample is determined using Sigma Diagnostics GPO-TRINDER enzymatic kit. The procedure outlined in the manual is adhered to, with the following exceptions: assay is performed in 48 well plate, 350 µL of sample volume was assayed, control blank consisted of 350 µL PBS/2% chaps, and standard contained 10 µL standard provide in kit plus 690 µL PBS/2% chaps. Analysis of samples is carried out on a Packard Spectra Count at a wavelength of 550 nm. Protein analysis is carried out on

25 μL of each supernatant sample using the BCA protein assay (Pierce) following manufacturer's instructions. Analysis of samples is carried out on a Packard Spectra Count at a wavelength of 550 nm.

In Vitro Glucose Uptake by Muscle Cells

L6 Muscle cells are obtained from the European Culture Collection (Porton Down) and are used at passages 7-11. Cells are maintained in standard tissue culture medium DMEM, and glucose uptake is assessed using [^{3}H]-2-deoxyglucose (2DG) with or without XCRF polypeptide fragment in the presence or absence of insulin (10^{-8} M) as has been previously described (Walker, P.S. et al. (1990) Glucose transport activity in L6 muscle cells is regulated by the coordinate control of subcellular glucose transporter distribution, biosynthesis, and mRNA transcription. JBC 265(3):1516-1523; and Kilp, A. et al. (1992) Stimulation of hexose transport by metformin in L6 muscle cells in culture. Endocrinology 130(5):2535-2544, which disclosures are hereby incorporated by reference in their entireties). Uptake of 2DG is expressed as the percentage change compared with control (no added insulin or XCRF polypeptide fragment). Values are presented as mean ± SEM of sets of 4 wells per experiment. Differences between sets of wells are evaluated by Student's t test, probability values $p<0.05$ are considered to be significant.

EXAMPLE 3: Effect of XCRF Polypeptides on Mice Fed a High-Fat Diet

Experiments are performed using approximately 6 week old C57Bl/6 mice (8 per group). All mice are housed individually. The mice are maintained on a high fat diet throughout each experiment. The high fat diet (cafeteria diet; D12331 from Research Diets, Inc.) has the following composition: protein kcal% 16, sucrose kcal% 26, and fat kcal% 58. The fat is primarily composed of coconut oil, hydrogenated.

After the mice are fed a high fat diet for 6 days, micro-osmotic pumps are inserted using isoflurane anesthesia, and are used to provide full-length XCRF polypeptides, XCRF polypeptide fragments, saline, and an irrelevant peptide to the mice subcutaneously (s.c.) for 18 days. XCRF polypeptides are provided at doses of 100, 50, 25, and 2.5 μg/day and the irrelevant peptide is provided at 10 μg/day. Body weight is measured on the first, third and fifth day of the high fat diet, and then daily after the start of treatment. Final blood samples are taken by cardiac puncture and are used to determine triglyceride (TG), total cholesterol (TC), glucose, leptin, and insulin levels. The amount of food consumed per day is also determined for each group.

EXAMPLE 4: Tests of Metabolic-related Activity in Humans

Tests of the efficacy of XCRF polypeptides in humans are performed in accordance with a physician's recommendations and with established guidelines. The parameters tested in mice are also

tested in humans (*e.g.* food intake, weight, TG, TC, glucose, insulin, leptin, FFA). It is expected that the physiological factors would show changes over the short term. Changes in weight gain might require a longer period of time. In addition, the diet would need to be carefully monitored. XCRF polypeptides, preferably XCRF polypeptides comprising the globular C-terminal C1q homology domain, would be given in daily doses of about 6 mg protein per 70 kg person or about 10 mg per day. Other doses would also be tested, for instance 1 mg or 5 mg per day up to 20 mg, 50 mg, or 100 mg per day.

EXAMPLE 5: Tests of Metabolic-related Activity in a Murine Lipoatrophic Diabetes Model

Previously, leptin was reported to reverse insulin resistance and diabetes mellitus in mice with congenital lipodystrophy (Shimomura et al. Nature 401:73-76 (1999); hereby incorporated herein in its entirety including any drawings, figures, or tables). Leptin was found to be less effective in a different lipodystrophic mouse model of lipoatrophic diabetes (Gavrilova et al Nature 403: 850 (2000); hereby incorporated herein in its entirety including any drawings, figures, or tables). The instant invention encompasses the use of XCRF polypeptides for reducing the insulin resistance and hyperglycaemia in this model either alone or in combination with leptin, the leptin peptide (US provisional application No 60/155,506), or other compounds. Assays include that described previously in Gavrilova et al. ((2000) Diabetes 49:1910-6; (2000) Nature 403:850) using A-ZIP/F-1 mice, except that XCRF polypeptides would be administered using the methods previously described in Example 3 (or Examples 6-8). The glucose and insulin levels of the mice would be tested, and the food intake and liver weight monitored, as well as other factors, such as leptin, FFA, and TG levels, typically measured in our experiments (see Example 3, above, or Examples 6-8).

EXAMPLE 6: Effect of XCRF Polypeptides on Plasma Free Fatty Acid in C57 BL/6 Mice

The effect of XCRF polypeptides on postprandial lipemia (PPL) in normal C57BL6/J mice is tested.

The mice used in this experiment are fasted for 2 hours prior to the experiment after which a baseline blood sample is taken. All blood samples are taken from the tail using EDTA coated capillary tubes (50 μL each time point). At time 0 (8:30 AM), a standard high fat meal (6g butter, 6 g sunflower oil, 10 g nonfat dry milk, 10 g sucrose, 12 mL distilled water prepared fresh following Nb#6, JF, pg.1) is given by gavage (vol.=1% of body weight) to all animals.

Immediately following the high fat meal, 25μg an XCRF polypeptide is injected i.p. in 100 μL saline. The same dose (25μg/mL in 100μL) is again injected at 45 min and at 1 hr 45 min. Control animals are injected with saline (3x100μL). Untreated and treated animals are handled in an alternating mode.

Blood samples are taken in hourly intervals, and are immediately put on ice. Plasma is prepared by centrifugation following each time point. Plasma is kept at –20°C and free fatty acids (FFA), triglycerides (TG) and glucose are determined within 24 hours using standard test kits (Sigma and Wako). Due to the limited amount of plasma available, glucose is determined in duplicate using pooled samples. For each time point, equal volumes of plasma from all 8 animals per treatment group are pooled.

EXAMPLE 7: Effect of XCRF Polypeptides on Plasma Leptin and Insulin in C57 BL/6 Mice

The effect of XCRF polypeptides on plasma leptin and insulin levels during postprandial lipemia (PPL) in normal C57BL6/J mice is tested. The experimental procedure is the same as that described in Example 6, except that blood was drawn only at 0, 2 and 4 hours to allow for greater blood samples needed for the determination of leptin and insulin by RIA.

Briefly, 16 mice are fasted for 2 hours prior to the experiment after which a baseline blood sample is taken. All blood samples are taken from the tail using EDTA coated capillary tubes (100 μL each time point). At time 0 (9:00AM), a standard high fat meal (see Example 6) is given by gavage (vol.=1% of body weight) to all animals. Immediately following the high fat meal, 25 μg of an XCRF polypeptide is injected i.p. in 100 μL saline. The same dose (25μg in 100μL) is again injected at 45 min and at 1 hr 45 min (treated group). Control animals are injected with saline (3x100μL). Untreated and treated animals are handled in an alternating mode.

Blood samples are immediately put on ice and plasma is prepared by centrifugation following each time point. Plasma is kept at –20°C and free fatty acids (FFA) are determined within 24 hours using a standard test kit (Wako). Leptin and Insulin are determined by RIA (ML-82K and SRI-13K, LINCO Research, Inc., St. Charles, MO) following the manufacturer's protocol; however, only 20 μL plasma is used. Each determination is done in duplicate. Due to the limited amount of plasma available, leptin and insulin are determined in 4 pools of 2 animals each in both treatment groups.

EXAMPLE 8: Effect of XCRF Polypeptides on Plasma FFA, TG and Glucose in C57 BL/6 Mice

The effect of XCRF polypeptides on plasma FFA, TG, glucose, leptin and insulin levels during postprandial lipemia (PPL) in normal C57BL6/J mice has been described. Weight loss resulting from XCRF polypeptides (2.5μg/day) given to normal C57BL6/J mice on a high fat diet has also been shown (Example 3).

The experimental procedure is similar to that described in Example 6. Briefly, 14 mice re fasted for 2 hours prior to the experiment after which a baseline blood sample is taken. All blood samples are taken from the tail using EDTA coated capillary tubes (50 μL each time point). At time 0 (9:00AM), a

standard high fat meal (see Example 6) is given by gavage (vol.=1% of body weight) to all animals. Immediately following the high fat meal, 4 mice are injected 25 μg of an XCRF polypeptide i.p. in 100μL saline. The same dose (25μg in 100μL) is again injected at 45 min and at 1 hr 45 min. A second treatment group receives 3 times 50 μg XCRF polypeptide at the same intervals. Control animals are injected with saline (3x100μL). Untreated and treated animals are handled in an alternating mode.

Blood samples are immediately put on ice. Plasma is prepared by centrifugation following each time point. Plasma is kept at –20 °C and free fatty acids (FFA), triglycerides (TG) and glucose are determined within 24 hours using standard test kits (Sigma and Wako).

EXAMPLE 9: Effect of XCRF Polypeptides on FFA following Epinephrine Injection

In mice, plasma free fatty acids increase after intragastric administration of a high fat/sucrose test meal. These free fatty acids are mostly produced by the activity of lipolytic enzymes *i.e.* lipoprotein lipase (LPL) and hepatic lipase (HL). In this species, these enzymes are found in significant amounts both bound to endothelium and freely circulating in plasma. Another source of plasma free fatty acids is hormone sensitive lipase (HSL) that releases free fatty acids from adipose tissue after β-adrenergic stimulation. To test whether XCRF polypeptides also regulate the metabolism of free fatty acid released by HSL, mice are injected with epinephrine.

Two groups of mice are given epinephrine (5μg) by intraperitoneal injection. A treated group is injected with an XCRF polypeptide (25μg) one hour before and again together with epinephrine, while control animals receive saline. Plasma is isolated and free fatty acids and glucose are measured as described above (Example 8).

EXAMPLE 10: Effect of XCRF Polypeptides on Muscle FFA Oxidation

To investigate the effect of XCRF polypeptides on muscle free fatty acid oxidation, intact hind limb muscles from C57BL/6J mice are isolated and FFA oxidation is measured using oleate as substrate (Clee, S. M. *et al.* Plasma and vessel wall lipoprotein lipase have different roles in atherosclerosis. *J Lipid Res* 41, 521-531 (2000); Muoio, D. M., Dohm, G. L., Tapscott, E. B. & Coleman, R. A. Leptin opposes insulin's effects on fatty acid partitioning in muscles isolated from obese ob/ob mice. *Am J Physiol* 276, E913-921 (1999)) Oleate oxidation in isolated muscle is measured as previously described (Cuendet et al (1976) J Clin Invest 58:1078-1088; Le Marchand-Brustel, Y., Jeanrenaud, B. & Freychet, P. Insulin binding and effects in isolated soleus muscle of lean and obese mice. *Am J Physiol* 234, E348-E358 (1978). Briefly, mice are sacrificed by cervical dislocation and soleus and EDL muscles are rapidly isolated from the hind limbs. The distal tendon of each muscle is tied to a piece of suture to facilitate transfer among different media. All incubations are carried out at 30°C in 1.5 mL of Krebs-

Henseleit bicarbonate buffer (118.6 mM NaCl, 4.76 mM KCl, 1.19 mM KH_2PO_4, 1.19 mM $MgSO_4$, 2.54 mM $CaCl_2$, 25mM $NaHCO_3$, 10 mM Hepes, pH 7.4) supplemented with 4% FFA free bovine serum albumin (fraction V, RIA grade, Sigma) and 5 mM glucose (Sigma). The total concentration of oleate (Sigma) throughout the experiment is 0.25 mM. All media are oxygenated (95% O_2; 5% CO_2) prior to incubation. The gas mixture is hydrated throughout the experiment by bubbling through a gas washer (Kontes Inc., Vineland, NJ).

Muscles are rinsed for 30 min in incubation media with oxygenation. The muscles are then transferred to fresh media (1.5 mL) and incubated at 30°C in the presence of 1μCi/mL [1-^{14}C] oleic acid (American Radiolabelled Chemicals). The incubation vials containing this media are sealed with a rubber septum from which a center well carrying a piece of Whatman paper (1.5 cm x 11.5 cm) is suspended.

After an initial incubation period of 10min with constant oxygenation, gas circulation is removed to close the system to the outside environment and the muscles are incubated for 90 min at 30°C. At the end of this period, 0.45 mL of Solvable (Packard Instruments, Meriden, CT) is injected onto the Whatman paper in the center well and oleate oxidation by the muscle is stopped by transferring the vial onto ice.

After 5 min, the muscle is removed from the medium, and an aliquot of 0.5 mL medium is also removed. The vials are closed again and 1 mL of 35% perchloric acid is injected with a syringe into the media by piercing through the rubber septum. The CO_2 released from the acidified media is collected by the Solvable in the center well. After a 90 min collection period at 30°C, the Whatman paper is removed from the center well and placed in scintillation vials containing 15 mL of scintillation fluid (HionicFlour, Packard Instruments, Meriden, CT). The amount of ^{14}C radioactivity is quantitated by liquid scintillation counting. The rate of oleate oxidation is expressed as nmol oleate produced in 90min/g muscle.

To test the effect of full-length XCRF polypeptide or XCRF polypeptide fragment comprising the globular C-terminal C1q homology domain on oleate oxidation, these proteins are added to the media at a final concentration of 2.5 μg/mL and maintained in the media throughout the procedure.

EXAMPLE 11: Effect of XCRF Polypeptides on Triglyceride in Muscle & Liver Isolated from Mice

To determine whether the increased FFA oxidation induced by XCRF polypeptides is also accompanied by increased FFA delivery into muscle or liver, the hindlimb muscle and liver triglyceride content is measured after the XCRF polypeptide treatment of mice. Hind limb muscles as well as liver samples are removed from treated and untreated animals and the triglyceride and free fatty acid

concentration is determined following a standard lipid extraction method (Shimabukuro, M. *et al.* Direct antidiabetic effect of leptin through triglyceride depletion of tissues. Proc Natl Acad Sci U S A 94:4637-4641 (1997)) followed by TG and FFA analysis using standard test kits.

EXAMPLE 12: Effect of XCRF Polypeptides on FFA following Intralipid Injection

Two groups of mice are intravenously (tail vein) injected with 30 µL bolus of Intralipid-20% (Clintec) to generate a sudden rise in plasma FFAs, thus by-passing intestinal absorption. (Intralipid is an intravenous fat emulsion used in nutritional therapy). A treated group (XCRF polypeptide-treated) is injected with an XCRF polypeptide (25µg) at 30 and 60 minutes before Intralipid is given, while control animals receive saline. Plasma is isolated and FFAs are measured as described previously. The effect of XCRF polypeptides on the decay in plasma FFAs following the peak induced by Intralipid injection is then monitored.

EXAMPLE 13: *In vitro* glucose uptake by muscle cells

L6 Muscle cells are obtained from the European Culture Collection (Porton Down) and are used at passages 7-11. Cells are maintained in standard tissue culture medium DMEM, and glucose uptake is assessed using [^{3}H]-2-deoxyglucose (2DG) with or without XCRF polypeptides in the presence or absence of insulin (10^{-8} M) as has been previously described (Walker, P.S. et al. (1990) Glucose transport activity in L6 muscle cells is regulated by the coordinate control of subcellular glucose transporter distribution, biosynthesis, and mRNA transcription. JBC 265:1516-1523; and Kilp, A. et al. (1992) Stimulation of hexose transport by metformin in L6 muscle cells in culture, Endocrinology 130:2535-2544, which disclosures are hereby incorporated by reference in their entireties). Uptake of 2DG is expressed as the percentage change compared with control (no added insulin or XCRF). Values are presented as mean ±SEM of sets of 4 wells per experiment. Differences between sets of wells are evaluated by Student's t test, probability values $p<0.05$ are considered to be significant.

EXAMPLE 14: *In Vivo* Tests for Metabolic-related Activity in Rodent Diabetes Models

As metabolic profiles differ among various animal models of obesity and diabetes, analysis of multiple models is undertaken to separate the effects XCRF polypeptides on hyperglycemia, hyperinsulinemia, hyperlipidemia and obesity. Mutations within colonies of laboratory animals and different sensitivities to dietary regimens have made the development of animal models with non-insulin dependent diabetes associated with obesity and insulin resistance possible. Genetic models such as db/db and ob/ob (See Diabetes, (1982) 31(1): 1-6) in mice and fa/fa in zucker rats have been developed by the various laboratories for understanding the pathophysiology of disease and testing the efficacy of

new antidiabetic compounds (Diabetes, (1983) 32: 830-838; Annu Rep Sankyo Res Lab (1994) 46: 1-57). The homozygous animals, C57 BL/KsJ-db/db mice developed by Jackson Laboratory, US, are obese, hyperglycemic, hyperinsulinemic and insulin resistant (J Clin Invest, (1990) 85: 962-967), whereas heterozygous are lean and normoglycemic. In db/db model, mouse progressively develops insulinopenia with age, a feature commonly observed in late stages of human type II diabetes when blood sugar levels are insufficiently controlled. The state of pancreas and its course vary according to the models. Since this model resembles that of type II diabetes mellitus, the compounds of the present invention are tested for blood sugar and triglycerides lowering activities. Zucker (fa/fa) rats are severely obese, hyperinsulinemic, and insulin resistant (Coleman, Diabetes 31:1, 1982; E. Shafrir, in Diabetes Mellitus; H. Rifkin and D. Porte, Jr. Eds. (Elsevier Science Publishing Co., Inc., New York, ed. 4, 1990), pp. 299-340), and the fa/fa mutation may be the rat equivalent of the murine db mutation (Friedman et al., Cell 69:217-220, 1992; Truett et al., Proc. Natl. Acad. Sci. USA 88:7806, 1991). Tubby (tub/tub) mice are characterized by obesity, moderate insulin resistance and hyperinsulinemia without significant hyperglycemia (Coleman et al., J. Heredity 81:424, 1990).

Previously, leptin was reported to reverse insulin resistance and diabetes mellitus in mice with congenital lipodystrophy (Shimomura et al. Nature 401: 73-76 (1999). Leptin is found to be less effective in a different lipodystrophic mouse model of lipoatrophic diabetes (Gavrilova et al Nature 403: 850 (2000); hereby incorporated herein in its entirety including any drawings, figures, or tables).

The streptozotocin (STZ) model for chemically-induced diabetes is tested to examine the effects of hyperglycemia in the absence of obesity. STZ-treated animals are deficient in insulin and severely hyperglycemic (Coleman, Diabetes 31:1, 1982; E. Shafrir, in Diabetes Mellitus; H. Rifkin and D. Porte, Jr. Eds. (Elsevier Science Publishing Co., Inc., New York, ed. 4, 1990), pp. 299-340). The monosodium glutamate (MSG) model for chemically-induced obesity (Olney, Science 164:719, 1969; Cameron et al., Clin Exp Pharmacol Physiol 5:41, 1978), in which obesity is less severe than in the genetic models and develops without hyperphagia, hyperinsulinemia and insulin resistance, is also examined. Finally, a non-chemical, non-genetic model for induction of obesity includes feeding rodents a high fat/high carbohydrate (cafeteria diet) diet *ad libitum*.

The instant invention encompasses the use of XCRF polypeptides for reducing the insulin resistance and hyperglycemia in any or all of the above rodent diabetes models or in humans with Type I or Type II diabetes or other prefered metabolic diseases described previously or models based on other mammals. In the compositions of the present invention the XCRF polypeptides may, if desired, be associated with other compatible pharmacologically active antidiabetic agents such as insulin, leptin (US provisional application No 60/155,506), or troglitazone, either alone or in combination. Assays include that described previously in Gavrilova et al. ((2000) Diabetes 49:1910-6; (2000) Nature

403:850) using A-ZIP/F-1 mice, except that XCRF polypeptides are administered intraperitoneally, subcutaneously, intramuscularly or intravenously. The glucose and insulin levels of the mice would be tested, and the food intake and liver weight monitored, as well as other factors, such as leptin, FFA, and TG levels, typically measured in our experiments.

In Vivo Assay for Anti-hyperglycemic Activity of XCRF polypeptides

Genetically altered obese diabetic mice (db/db) (male, 7-9 weeks old) are housed (7-9 mice/cage) under standard laboratory conditions at 22° C and 50% relative humidity, and maintained on a diet of Purina rodent chow and water *ad libitum*. Prior to treatment, blood is collected from the tail vein of each animal and blood glucose concentrations are determined using One Touch Basic Glucose Monitor System (Lifescan). Mice that have plasma glucose levels between 250 to 500 mg/dl are used. Each treatment group consists of seven mice that are distributed so that the mean glucose levels are equivalent in each group at the start of the study. db/db mice are dosed by micro-osmotic pumps, inserted using isoflurane anesthesia, to provide XCRF polypeptides, saline, and an irrelevant peptide to the mice subcutaneously (s.c.). Blood is sampled from the tail vein hourly for 4 hours and at 24, 30 h post-dosing and analyzed for blood glucose concentrations. Food is withdrawn from 0-4 h post dosing and reintroduced thereafter. Individual body weights and mean food consumption (each cage) are also measured after 24 h. Significant differences between groups (comparing XCRF treated to saline-treated) are evaluated using Student t-test.

In Vivo Insulin Sensitivity Assay

In vivo insulin sensitivity is examined by utilizing two-step hyperinsulinemic-euglycemic clamps according to the following protocol. Rodents from any or all of the various models described in Example 2 are housed for at least a week prior to experimental procedures. Surgeries for the placement of jugular vein and carotid artery catheters are performed under sterile conditions using ketamine and xylazine (i.m.) anesthesia. After surgery, all rodents are allowed to regain consciousness and placed in individual cages. XCRF polypeptides or vehicle is administered through the jugular vein after complete recovery and for the following two days. Sixteen hours after the last treatment, hyperinsulinemic-euglycemic clamps are performed. Rodents are placed in restrainers and a bolus of 4 µCi [3-^{3}H] glucose (NEN) is administered, followed by a continuous infusion of the tracer at a dose of 0.2 µCi/min (20 µl/min). Two hours after the start of the tracer infusion, 3 blood samples (0.3 ml each) are collected at 10 minute intervals (-20-0 min) for basal measurements. An insulin infusion is then started (5 mU/kg/min), and 100 µl blood samples are taken every 10 min. to monitor plasma glucose. A 30% glucose solution is infused using a second pump based on the plasma glucose levels in order to reach

and maintain euglycemia. Once a steady state is established at 5 mU/kg/min insulin (stable glucose infusion rate and plasma glucose), 3 additional blood samples (0.3 ml each) are obtained for measurements of glucose, [3-^{3}H] glucose and insulin (100-120 min.). A higher dose of insulin (25 mU/kg/min.) is then administered and glucose infusion rates are adjusted for the second euglycemic clamp and blood samples are taken at min. 220-240. Glucose specific activity is determined in deproteinized plasma and the calculations of Rd and hepatic glucose output (HGO) are made, as described (Lang et al., Endocrinology 130:43, 1992). Plasma insulin levels at basal period and after 5 and 25 mU/kg/min. infusions are then determined and compared between XCRF treated and vehicle treated rodents.

Insulin regulation of glucose homeostasis has two major components; stimulation of peripheral glucose uptake and suppression of hepatic glucose output. Using tracer studies in the glucose clamps, it is possible to determine which portion of the insulin response is affected by the XCRF polypeptides.

EXAMPLE 15: Effect of XCRF Polypeptides on Weight Gain and Weight Loss of Mice and on Maintenance of Weight Loss in Mice

In the first experiment, 10-week-old male C57BL/6J mice are put on a very high fat/sucrose purified diet for 19 days to promote weight gain (see Example 3); the average body weight at this time is 30g. The mice are then surgically implanted with an osmotic pump (Alzet, Newark, DE) delivering either 2.5 µg/day of XCRF polypeptide fragment comprising all or part of the globular C-terminal C1q homology domain, 5µg/day of full-length XCRF, or physiological saline. The mice are continued on the high fat diet and their body weight was recorded over the following 10-day period.

Weight gain by mice treated with saline in contradistinction to weight loss by mice treated either with XCRF polypeptide fragment or XCRF full-length polypeptide is taken as evidence that in this inbred strain of normal mice, a continuous infusion of a daily low dose of XCRF polypeptide fragment or XCRF full-length polypeptide can prevent weight gain caused by high fat/sucrose feeding, in a sustainable way.

Data are expressed throughout as mean ± SEM; a p-value < 0.05 is considered statistically significant. Statistical analysis is typically done using either the unpaired Student's t test or the paired Student's t test.

Maintenance of weight loss in mice

In order to demonstrate the ability of XCRF polypeptide to maintain weight loss, normal mice are put on a reduced calorie diet to promote weight loss. The reduced calorie diet is continued until the mice lose 10% of their initial weight. A second group of mice are continued on the reduced calorie diet

until the mice lose 20% of their initial weight. The mice are then surgically implanted with an osmotic pump (Alzet, Newark, DE) delivering either 2.5 μg/day of XCRF polypeptide fragment comprising all or part of the globular C-terminal C1q homology domain, 5μg/day of XCRF full-length polypeptide, or physiological saline. The mice are returned to a normal diet and their body weights are recorded over a 10-day period. After 10 days, the outcome wherein mice treated with XCRF polypeptide fragment or XCRF full-length polypeptide have a lower weight than mice treated with saline is taken to provide evidence that treatment with XCRF polypeptide fragment or XCRF full-length polypeptide promotes the maintenance of weight loss.